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DIFFERENT CHROMOMERE NUMBERS AT MEIOSIS AND MITOSIS IN ORNITHOGALUM

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(Received February 25th, 1959)

INTRODUCTION

THE chromomere number has been considered a constant feature of a species, an idea which sprang from BELLING's analysis of chromomeres in flowering plants and from his association of the chromomere with the gene. In *Drosophila* PAINTER, BRIDGES and others associated the gene with the bands of salivary gland chromosomes by ascribing definite genes to identifiable bands. The number and morphology of bands was found to be most constant for *Drosophila* salivary chromosomes and as a consequence, gene and band and gene and chromomere were understood to be directly related entities, the constancy of one taken to imply the constancy of the other.

The measurements of DNA content per nucleus made by michrophotometric methods apparently contributed to reinforce this concept. The amount of DNA was found to be constant per set of chromatids in nuclei from different tissues of the same species (BOIVIN, VENDRELY and VENDRELY, 1948, MIRSKY and RIS, 1949, PATAU and SWIFT, 1953 and others). These results contained, however, limitations inherent in the techniques employed.

Recent studies on DNA content have shown that certain regions of the same chromosome may show different amounts of DNA. In *Glyptotendipes* (Diptera) the large puffed regions of the middle of each of the three chromosomes show an 8-fold increase in DNA when larvae are grown at 18° instead of at 8°C (STICH and NAYLOR, 1958). In *Rhynchosciara* there is also a high local incorporation of thymidine in certain regions of the giant chromosomes. This incorporation varies at different stages of larval life (FICQ and PAVAN, 1957). In the same orgauism RUDKIN and CORLETTE (1957) found that the DNA in puffed regions doubles with respect to non-puffing regions during the development of the larvae.

Also in Diptera it has recently been shown that the same band or group of bands may have a different structure in different tissues and that this variation is related to the specialized physiological activity of the cell (BEERMANN, 1952, PAVAN and BREUER, 1952). There is also an indication that the total number of bands of a chromosome varies from tissue to tissue (BEERMANN, 1956).

In *Agapanthus umbellatus* the chromosomes show different chromomere numbers in different tissues (LIMA-DE-FARIA, 1954). However, in this species individual chromosomes could not be readily identified and the chromomere structure studied at stages other than pachytene was not as distinct as at pachytene. In *Ornithogalum virens*, a plant with only three chromosomes, each chromosome can be identified at most stages and the chromomere structure found at the second microspore prophase is strikingly similar to that seen at pachytene. For this reason a study of this species was carried out with a view to analyzing the structural variation of each chromosome in different tissues and at different stages.

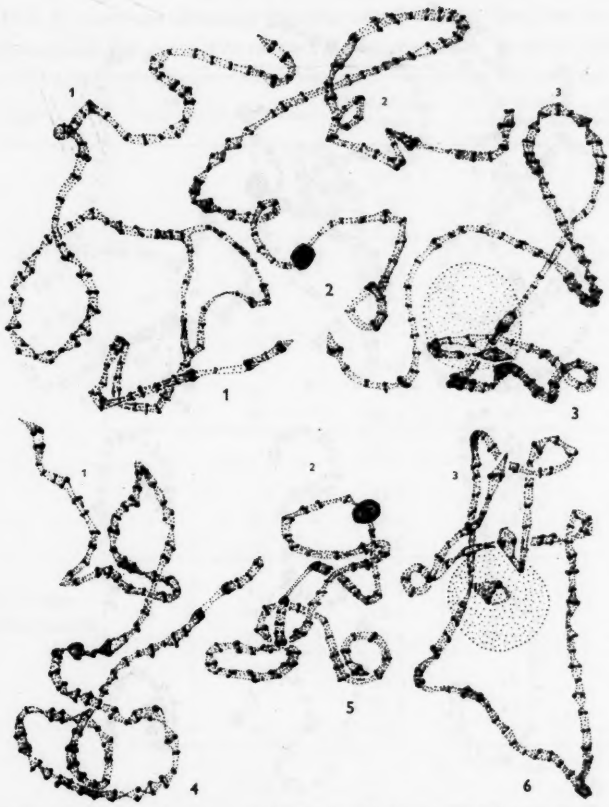
MATERIAL AND METHODS

Root tips of *O. virens* (n=3) were stained with aceto-orcein after fixation in acetic-alcohol 1 : 3, or with fuchsin-sulphurous acid after fixation in acetic-alcohol 1 : 3 (1 minute) followed by Nawashin-Karpechenko for 30 minutes. Stages of meiosis and microspore mitosis were stained with aceto-carmine after fixation in acetic-alcohol 1 : 4. All figures are camera lucida drawings of permanent preparations.

Although *O. virens* has only three bivalents at pachytene, these are difficult to analyze due to their length. The chromosomes are entangled with each other in a nucleus and cell too small for them. In well-squashed cells whole chromosomes can often be analyzed, but cells where the whole complement can be followed are more difficult to find.

THE CHROMOMERE NUMBER AT MEIOSIS AND MITOSIS

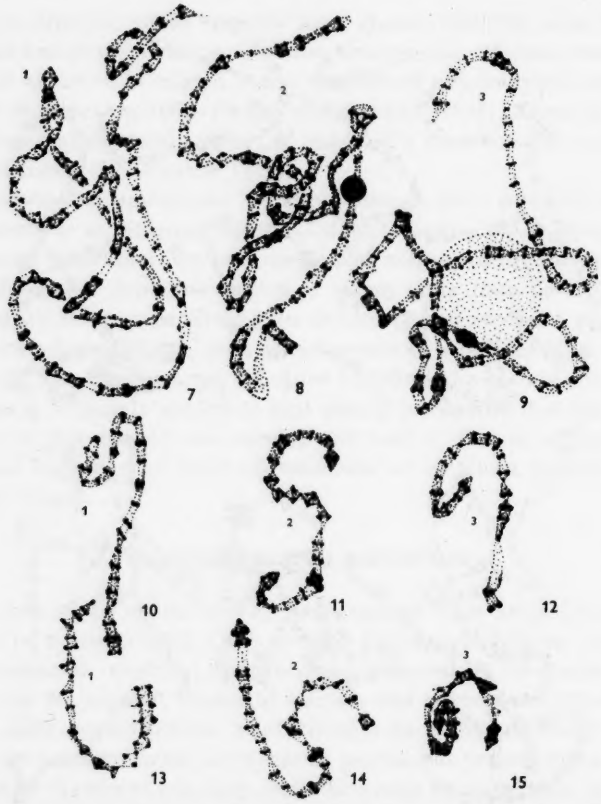
At pachytene the three chromosomes are distinguished from each other by the following main features: chromosome I has no knob, chromosome II has a large knob and chromosome III is the nucleolar chromosome. Moreover, each chromosome has a particular sequence of chromomeres at the ends that helps in its identification (Figs. 1—9). There are no gradients in these chromosomes.



Figs. 1—6. The three chromosomes at pachytene in two cells. The small figures refer to the chromosome number given in the text. — ×2300.

In *O. virens* there is another stage where chromomeres are very clear, and an inexperienced observer may believe at first that he is observing pachytene chromosomes. This is the prophase of the second microspore mitosis. This prophase takes place within the pollen grain, whereas in other species of the Liliaceae it takes place in the pollen tube. The long duration of this prophase may account for the detailed structure displayed.

The three chromosomes are more difficult to identify at this stage since the knob is smaller and sometimes not easily seen, and the nucleolus is not readily distinguishable. However, in cells where the



Figs. 7—15. Comparison between the three chromosomes at pachytene (Figs. 7—9) and at the second microspore prophase (Figs. 10—12, one cell, and 13—15 another cell). Chromosome I (Figs. 7, 10, 13), chromosome II (Figs. 8, 11, 14), chromosome III (Figs. 9, 12, 15). At the microspore prophase each chromosome is composed of two chromatids; at pachytene each bivalent consists of two chromosomes. — ×2300.

knob is recognizable, chromosome I is distinguished from III by being larger and lacking the weakly stained zone that is a characeristic feaure of III (Figs. 10—15).

In Table I a comparison is made in three cells, where the whole complement could be analyzed, between pachytene and microspore prophase chromosomes. The same chromosome is four to five times shorter at the microspore mitosis than at pachytene, but its chromomere number is also about four times lower. The chromomere number

TABLE 1. *Average distance in microns between two consecutive chromomeres in the cells where every chromosome could be identified.*

Stage	Cell	Chromosome	Length in microns	Chromomere number	Average distance
Pachytene	1	I	110.0	111	0.99
		II	95.7	86	1.11
		III	97.1	90	1.08
	2	I	108.6	102	1.06
		II	95.7	84	1.14
		III	97.1	92	1.06
	3	I	108.6	103	1.05
		II	75.7	72	1.05
		III	101.4	91	1.11
				Mean:	1.07
Prophase of the second microspore mitosis	1	I	25.7	25	1.03
		II	23.1	24	0.96
		III	22.0	20	1.10
	2	I	21.4	27	0.79
		II	18.6	19	0.98
		III	20.6	23	0.90
	3	I	20.0	21	0.95
		II	27.1	27	1.00
		III	19.4	21	0.92
				Mean:	0.96

diminishes proportionately to the length in every chromosome. The average distance between consecutive chromomeres is of the same order of magnitude at both stages.

If the P. M. C.'s are well flattened, the total chromomere number of each cell can be accurately determined. In Table 2 the total chromosome length and chromomere number are given for 10 cells at pachytene and at the second microspore mitosis. The average chromomere number of the three chromosomes at these stages is 274.2 and 67.4, respectively, and the length 293.1 and 60.2 microns, respectively. The chromomere number follows closely the decrease in length. The average distance between two consecutive chromomeres is again of the same magnitude at both stages, but slightly lower at the microspore prophase.

TABLE 2. *Average distance in microns between two consecutive chromomeres at pachytene and prophase of the second microspore mitosis. Total analysis of the chromosome complement.*

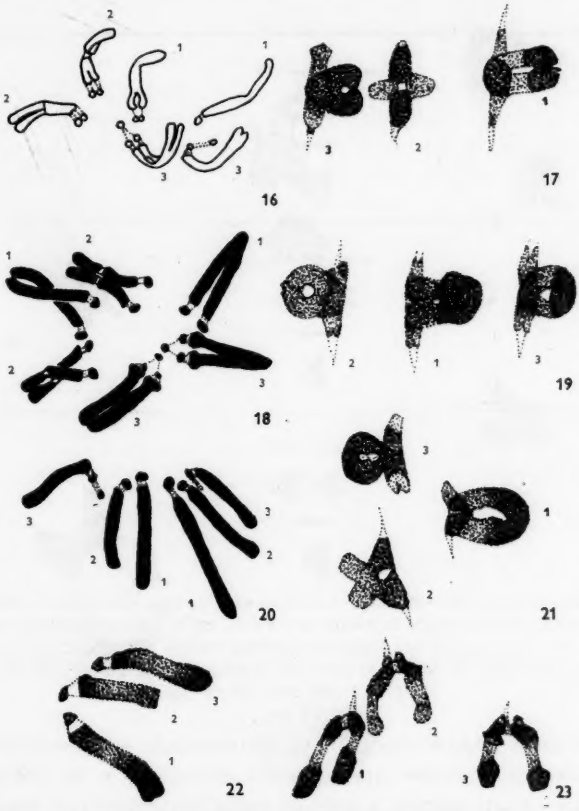
Cell	Pachytene			Prophase of the second microspore mitosis		
	Length in microns	Chromomere number	Average distance	Length in microns	Chromomere number	Average distance
1	302.8	287	1.06	70.8	68	1.03
2	301.4	278	1.08	60.6	69	0.88
3	285.7	266	1.07	60.7	78	0.78
4	261.4	233	1.12	66.5	69	0.96
5	262.8	275	0.96	59.4	67	0.89
6	274.3	270	1.02	48.0	63	0.76
7	302.9	305	0.99	85.4	74	1.15
8	274.3	265	1.04	52.9	63	0.84
9	314.3	286	1.10	43.1	57	0.76
10	351.4	277	1.27	54.9	65	0.84
Total	2.931.3	2.742		602.3	674	
Mean	293.1	274.2	1.07	60.2	67.4	0.89

COMPARISON OF THE SAME CHROMOSOME AT DIFFERENT STAGES

At metaphase of the root tip mitosis the three chromosomes, which have subterminal kinetochores, may be readily identified. Chromosome I is the longest, II shows a secondary constriction and III is the nucleolar chromosome with its small satellite in the short arm (Figs. 16 and 18). At anaphase the chromosomes are also distinguishable (Fig. 20).

At diakinesis (Figs. 24, 25) the chromosomes are easily identifiable: I is the longest, with both distal regions positively heteropycnotic; the same is the case for chromosome III which, in addition, forms the nucleolus; and II has only one distal region positively heteropycnotic.

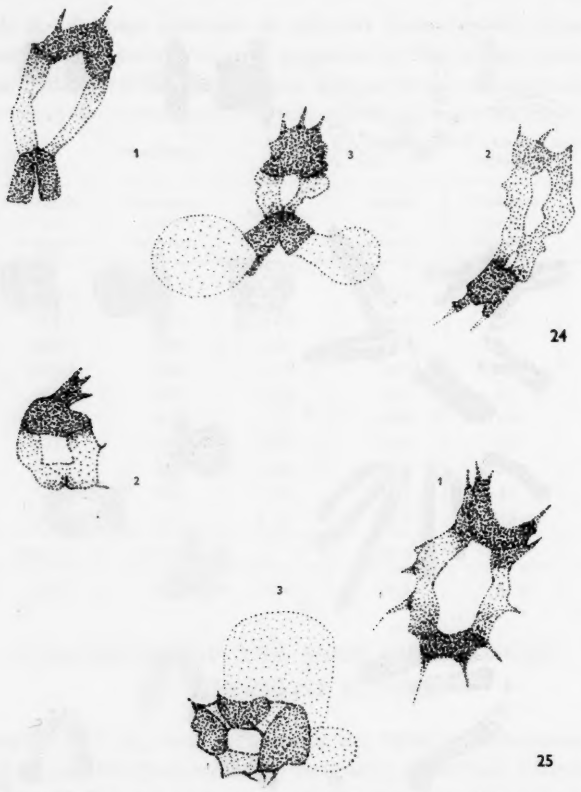
The appearance at diakinesis is the key to the identification at metaphase I (Figs. 17, 19 and 21). Here the nucleolus is no longer seen but the heteropycnotic regions are clearly distinguishable: I is the largest chromosome, forming as a rule two or three chiasmata; chromosome II is distinguished from III by the positively heteropycnotic distal region in the long arm of III. The same marks permit identification at anaphase I and anaphase II (Figs. 23 and 22, respectively).



Figs. 16—23. Metaphase of mitosis (Figs. 16 and 18) and anaphase of mitosis in root tips (Fig. 20). Three cells at metaphase I (Figs. 17, 19, 21). Anaphase I (Fig. 23) and anaphase II (Fig. 22). — ×2300.

In Figs. 26—40 the three chromosomes are drawn in the same scale at different stages.

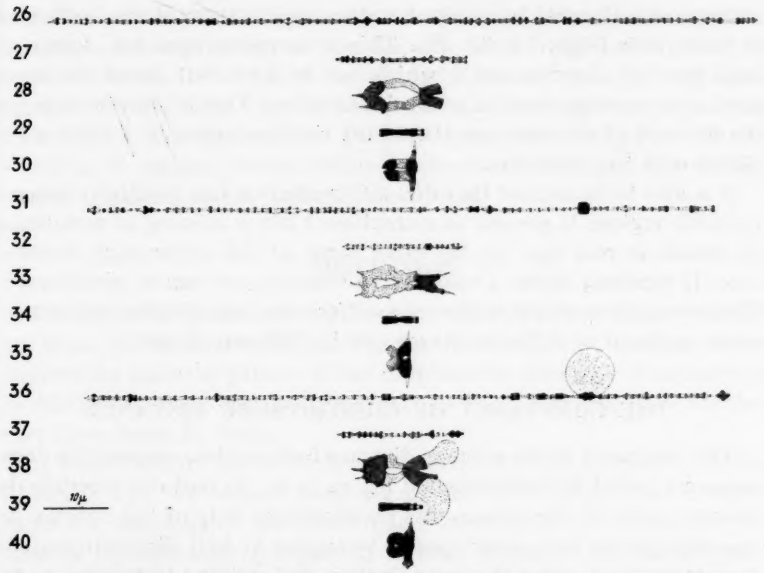
It is evident that a mechanical interpretation of the condensation process of the chromosomes does not fit the data. On the basis of such an interpretation the chromosomes at the second microspore prophase would show the same number of chromomeres as were present at pachytene but packed together much more closely. Instead the number of chromomeres in the microspore prophase is only one fourth that which is seen at pachytene, but the distance separating them is the



Figs. 24—25. The three chromosomes at diakinesis in two cells. — ×2300.

same in both cases. Moreover, the thickness of the two chromosomes at pachytene is essentially the same as that of the two chromatids at the microspore prophase. On the same grounds, by simple contraction, the knob of chromosome II would be much larger at the microspore prophase than at pachytene. The contrary is observed. Sometimes it is difficult to recognize the knob in the microspore division whereas at pachytene it is always visible as a large block of heterochromatin.

If one compares chromosome II at microspore prophase and at diakinesis (Figs. 32, 33) it may be seen that it has about the same length at the two stages but it shows a quite different organization. In the first one there is a distinct chromomere pattern; in the second, a conti-



Figs. 26—40. Comparisons among chromosome I (Figs. 26—30), chromosome II (Figs. 31—35) and chromosome III (Figs. 36—40) at different stages and in different tissues. Pachytene (Figs. 26, 31, 36), second microspore prophase (Figs. 27, 32, 37), diakinesis (Figs. 28, 33, 38), metaphase of mitosis in roots (Figs. 29, 34, 39) and metaphase I (Figs. 30, 35, and 40). — ×1000.

nuous structure. The chromosome at diakinesis results from the direct condensation of a pachytene chromosome, whereas the microspore chromosome is reorganized after passing a resting stage — as in the case of a pachytene chromosome. During the resting stage, when the chromosomes are swollen and elongated, the basic structural relations within the chromosome are determined for the following division.

Comparison of the same chromosome at diakinesis and the microspore prophase shows clearly that the chromomere structure observed at the latter stage is not the product of any optical effect. In one case the chromomeres are distinctly visible; in the other they are not. Moreover, the resolving power of the light microscope is of the order of 0.3 microns and at the microspore prophase, as at pachytene, the chromomere centers are separated by three times this distance, and the actual interchromomere distance is twice the resolving power value.

Another interesting feature is the positively heteropycnotic region seen at diakinesis and subsequent stages of meiosis. The dark region in

chromosome II could be ascribed to the condensation of the knob seen at pachytene (Figs. 31, 32, 33). This assumption does not, however, hold true for chromosome I, which has no knob but shows the same degree of heteropycnosis at both distal regions. This is also the case for the left end of chromosome III. At the right end there is a knob associated with the nucleolus.

It is also to be noticed that this differentiation into positively heteropycnotic regions is present at metaphase I but is missing at metaphase of mitosis in root tips. On the other hand, at this latter stage chromosome II regularly shows a constriction which is not seen at metaphase I. This is one more manifestation of a differential organization of chromosome segments at different stages and in different tissues.

THE CONSTANCY OF CHROMOMERE DISTANCE

The constancy of the average distance between two consecutive chromomeres found in *Ornithogalum* led us to try to find out whether the average value of one micron was characteristic only of this species. An investigation of four other species belonging to four different families was undertaken, using the same fixation and staining techniques as for *O. virens*. The values shown in Table 3 are based on the analysis of the whole chromosome complement for each stage indicated. The number of chromosomes analyzed varied from 15 to 35 for each stage. The

TABLE 3. *Average chromomere distance in different species.*
(For the number of chromosomes analyzed and the degree of variation see text.)

Family	Species	Stage	Average chromomere distance in microns
Liliaceae	*Ornithogalum virens*	Pachytene	1.07
	„ „	Second microspore prophase	0.89
Liliaceae	*Agapanthus umbellatus*	Pachytene	0.84
	„ „	Prophase II	0.95
	„ „	Prophase root tips	1.09
Gramineae	*Secale cereale*	Pachytene	0.82
Solanaceae	*Solanum lycopersicum*	Pachytene	0.83
	„ „	Prophase root tips	0.96
Labiatae	*Salvia horminum*	Pachytene	1.06
	„ „	Second microspore prophase	0.94
		Mean	0.95

variation encountered along the chromosome and among different chromosomes is insignificant as can be seen from Tables 4, 5 and 6 in LIMA-DE-FARIA (1954) where the measurements are given for very small arms containing only 3 to 4 chromomeres and for long arms containing as much as 54 chromomeres. Table 3 shows that, irrespective of the variation in species, family, tissue or chromosome length, the average distance remains on the order of one micron.

DISCUSSION

The results of BEERMANN (1952, 1956) and PAVAN and BREUER (1952) are those that best resemble the situation found in *Ornithogalum*. In the Diptera the essential pattern of the prophase chromosome is maintained in different tissues, but the appearance of the bands and their number vary from tissue to tissue.

In *Ornithogalum* the same situation occurs for mid prophase I of the P. M. C.'s and the second prophase of the microspore. The essential pattern is also kept — the chromomere structure is very clear and the knob of chromosome II is present in both tissues — but the chromomere number is quite different and the knob is distinctly smaller when the chromosome is shorter.

The situation is more extreme in *Ornithogalum* concerning the chromomere number and more extreme in the Diptera in regard to the variation in morphology of single segments. In the Diptera the variation in band number may range from 380 bands in the salivary glands to 480 in the interior portion of the midgut in *Chironomus tentans* — an increase of 100 (BEERMANN, 1956). This variation, however, involves mainly the faintest bands. One would expect that the variation in band number would not be very great in the Diptera since the chromosomes do not vary as much in length in the tissues studied as they do in *Ornithogalum*. On the other hand, the variation in the morphology of single segments is found to be more extreme in the Diptera, most probably due to the bands being much larger than the chromomeres. Changes occurring in them are easier to detect.

What is essential is that, although the pattern is maintained both in the Diptera and Ornithogalum, definite segments of the chromosome reorganize in different ways in different tissues. This is taken to mean that different genes are active at different stages of development. Since chromomeres are known to be tight spirals (RIS, 1957, EBERLE, 1957) different chromomere numbers represent a differential spiralization along

the chromosome body. As in the case of single bands that expand into puffs (where the surface available for reaction is strongly increased), single chromomeres, as opposed to interchromomere spaces, represent regions where the genes are more concealed in their activity. By differential spiralization along the chromosome body in different tissues different genes would get exposed or concealed at different stages of development.

The significance of the one micron distance is difficult to understand at present. But the existence of chromomeres as opposed to a continuous structure is already in itself a manifestation of periodicity. The similarity of the distance in different organisms and tissues is a still more concrete expression of this periodicity. It may simply be the microscopic expression of the periodicity imposed by the length or constitution of the DNA molecule.

MAZIA (1954) by appropriate treatment obtained fragmentation of the chromosomes. The significant point in his experiments was that the numerous segments into which the chromosome falls apart were found to be of similar size, their length being circa 0.4 microns (4,000 Å). This value is roughly half the length of the interchromomere distance. Although a precise homology between MAZIA's and the present findings cannot yet be established, both results demonstrate the same phenomenon: periodicity in the organization of the chromosome.

The rule of DNA constancy was established mainly by analysis of resting nuclei but the error involved in the measurements was sufficiently large to conceal small but specific changes. A variation in chromomere number may mean a small variation in DNA which only more refined methods will be able to detect.

SUMMARY

(1) In *Ornithogalum virens* the three chromosomes of the complement can be identified at all stages so far investigated.

(2) The same type of chromomere pattern is found at pachytene and at the second microspore prophase. The three chromosomes are four to five times shorter at the pollen mitosis than at pachytene but their chromomere number is also approximately four times lower. The chromomere number diminishes proportionately to the length in every chromosome. The average distance between two consecutive chromomeres is circa 1 micron at both stages.

(3) The analysis of the whole chromosome complement of four other

species belonging to the *Liliaceae*, *Gramineae*, *Solanaceae* and *Labiatae* reveals that, irrespective of the variation in species, family, tissue or chromosome length, the average chromomere distance remains on the order of 1 micron. This is evidence of a periodicity in the organization of the chromosome.

(4) Chromosome II has a large knob at pachytene that stands as a very distinct heterochromatic block. Contrary to the expectation of a simple mechanical process of condensation, this knob is very small and sometimes difficult to distinguish in chromosome II when it becomes one fourth shorter at the microspore prophase. Chromosome I, which has no knob or any other large chromomeres at pachytene, shows two large positively heteropycnotic segments at diakinesis and metaphase I. In the same chromosome this differentiation into heteropycnotic segments is missing at metaphase of mitosis in root tips. The variation in morphology at the knob region, the differential behavior of the heteropycnotic regions, and the difference in chromomere number are evidence that the same segments of the chromosome are organized in different ways in different tissues. These findings have direct implications for gene activity at different stages of development.

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NEO-XY MECHANISM IN TWO SCARABAEOID BEETLES, *PHANAEUS VINDEX* MacL. (SCARABAEIDAE), AND *DORCUS PARALLELOPIPEDUS* L. (LUCANIDAE)

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THE so-called neo-XY mechanism of sex chromosomes is represented by approximately 8—9 % of the cytologically known Coleoptera (SMITH 1953). The present article is a report of the first case of neo-XY encountered in the large coleopteran family Scarabaeidae, of which 75 species have been studied up to now. At the same time, a corresponding case from the related, cytologically little known family Lucanidae will be described.

The material comprises one specimen of the North American scarab, *Phanaeus vindex* MACL. (=*carnifex* (L)), and one European lucanid, *Dorcus parallelopipedus* L. Many thanks are due to Dr. HENRY F. HOWDEN (Div. of Entomology, Dept. of Agriculture, Ottawa, Canada) for collecting, fixing and dispatching *Phanaeus* material to me. Altogether 4 males were sent, but only one of them contained the desired stages of spermatogenesis. This specimen was collected in May, 1956, from the surroundings of Knoxville, Tenn.; the fixation was Carnoy. The only *Dorcus* specimen was captured by me in 1952 in Campdevánol, a village in the province of Gerona, northern Spain, and fixed in BAUER's modification of Boúin-Allen. After fixation, the testes of these two specimens were embedded in paraffin and sectioned in the usual way.

I. PHANAEUS VINDEX MACL.

The chromosome complement of this coprine scarab has been described by HAYDEN (1925) in a detailed study chiefly concerning the early prophase of male meiosis. Her count, 2n=12, is confirmed here.

This had long been the lowest known chromosome number in the Coleoptera, until PIZA (1958) recently announced 2n=10 in a South American elaterid. HAYDEN did not distinguish the sex chromosomes.

HAYDEN describes a peculiar history of thickening and fusion of the distal and then of the proximal chromosome regions to form a structure which she calls a "lantern figure". Related formations, due to the association of distally and proximally located heterochromites, have been reported by me in some other scarabs (VIRKKI 1951, 1954). The resolution of the "lantern" takes place, according to HAYDEN, at the beginning of diplotene, when the distal regions of the bivalents separate, and both these and the bivalent arms lose their stainability. The structure which remains visible is called the "karyosphere" by HAYDEN. In the reinvestigation, there appeared to be a compound chromocentre containing the heterochromatic procentric regions of the chromosomes (bivalents). HAYDEN's descriptions of the early prophase fit generally very well with the view gained by me from the specimen I have studied. I cannot, however, confirm her subsequent statements: 1) that the pairing of homologues takes place during the "lantern" stage, and 2) that the "lantern" is a regular formation in the prophase. Although there are often joined thickenings in the distal chromosome ends, I have never encountered complete, nicely formed "lantern figures". The pairing process, on the other hand, is presumably a very early process, as compared with the "lantern" stage. The interrelations between chromosome conjugation and the simultaneous heterochromatin associations would perhaps be interesting, but squashed material would be needed for such a study.

The arrangement of the bivalents in the forming "karyosphere" nucleus is distinctly regular and directly derived from the "lantern" stage. After some disintegration of the densely staining chromocentre, the thickened proximal regions of the individual bivalents can be discerned (Figs. 1 d, 3 c, 5 a). These interrelations as such concern 5 bivalents only. The sixth bivalent takes a differential position in about one half of the cases. It then lies at the "bottom" of the loose bouquet (Fig. 4 a—d). Even at this stage, a clearly recognizable thickening marks one of its ends (Fig. 1 e). This is, in short, the earliest observation of the form and behaviour of the neo-XY in the spermatogenesis of this species.

It is to be suspected that the distal end association of HAYDEN's "lantern" is better formed in cases where the neo-XY lies at the bottom of the bouquet. If it takes part in the "karyosphere" formation, its

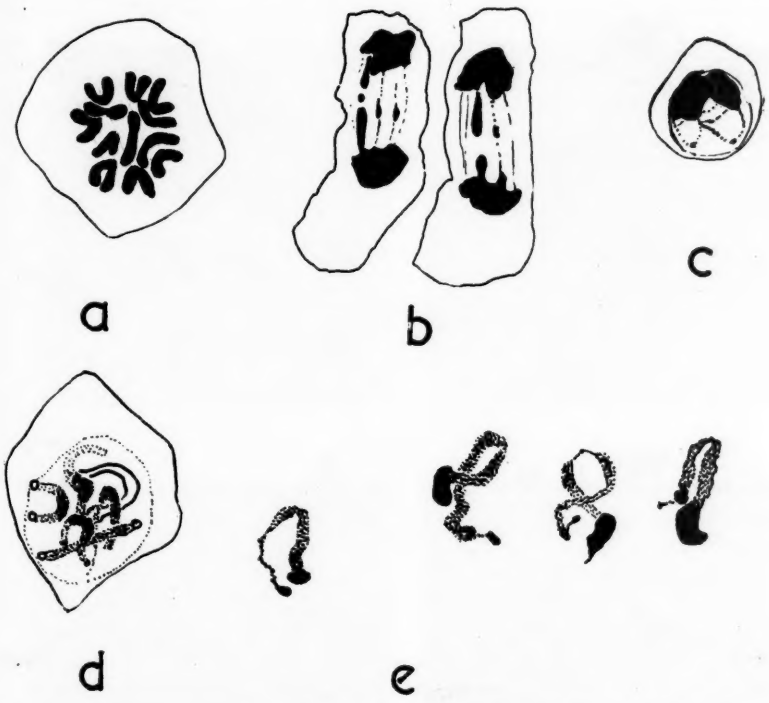

Fig. 1, a—e. *Phanaeus vindex*. — a. Spermatogonial metaphase, 2n=12. b. Spermatogonial anaphases, showing finger-like lagging chromosomes and granules of elimination chromatin. c. A very young 1st spermatocyte. d. Early diplotene, showing the beginnings of loss of stainability. Only outlines of the deeper-lying neo-XY have been drawn. e. Four neo-XY's of a similar stage to d. — ×2480.

other regions rather than the distal knob fuse with the proximal regions of the autosome bivalents. The frequent differential position of the neo-XY is often also recognizable in the nuclei of the most advanced diffusion, owing to the heterochromasy of the knob, or former X chromosome.

Like other scarabs with a diffuse diplotene, *Phanaeus vindex* shows a very rapid reappearance of the euchromatin at the beginning of diakinesis. Nuclei with bivalents in a diffuse condition, and others with well formed diakinesis bivalents can be found in one and the same cyst. On reappearing, the euchromatic segments of the autosomes are at first very thin, their double structure being hardly recognizable. They are

Fig. 2, a—e. *Phanaeus vindex*. — a—d. Early diakinetic bivalents at the stage of rapid reappearance of the stainability and disintegration of the compound chromocentre. e. Diakinesis. — Every row shows the whole contents of a nucleus. Neo-XY at right, the still persisting association of 2—4 bivalents to the left. — ×2480.

of approximately the same length as at the formation of the "karyosphere", so that there is hardly any difference in the degree of spiralization. The homologous chromosomes of a bivalent have withdrawn from one another despite the fact that the procentric heterochromites keep the centromeres together. The association of the bivalents by means of the procentric heterochromatin is breaking down in this phase (Figs. 2, 3 d). Even at this early stage of tetrad history, the chiasmata

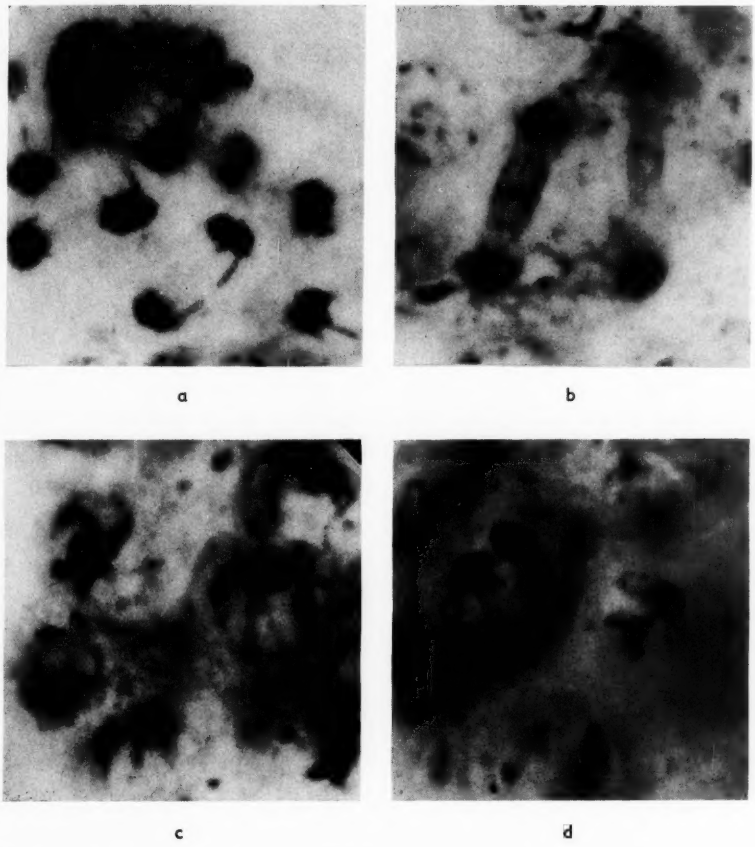

Fig. 3, a—d. *Phanæus vindex*. — a. Middle, b. late spermatogonial anaphases, showing the lagging chromosomes (neo-X and neo-Y?) and elimination chromatin (comp. with Fig. 1 b). — c. Side and polar views of early diplotene at the same stage as in Fig. 1 d. Neo-XY in opposite position to the compound chromocentre, except in the nucleus situated middle right, where it takes part in the chromocentre. — d. Diakinesis. Stage of the rapid reappearance of stainability and distintegration of the chromocentre (as in Fig. 2, a—d). Hematoxylin. — ×4640.

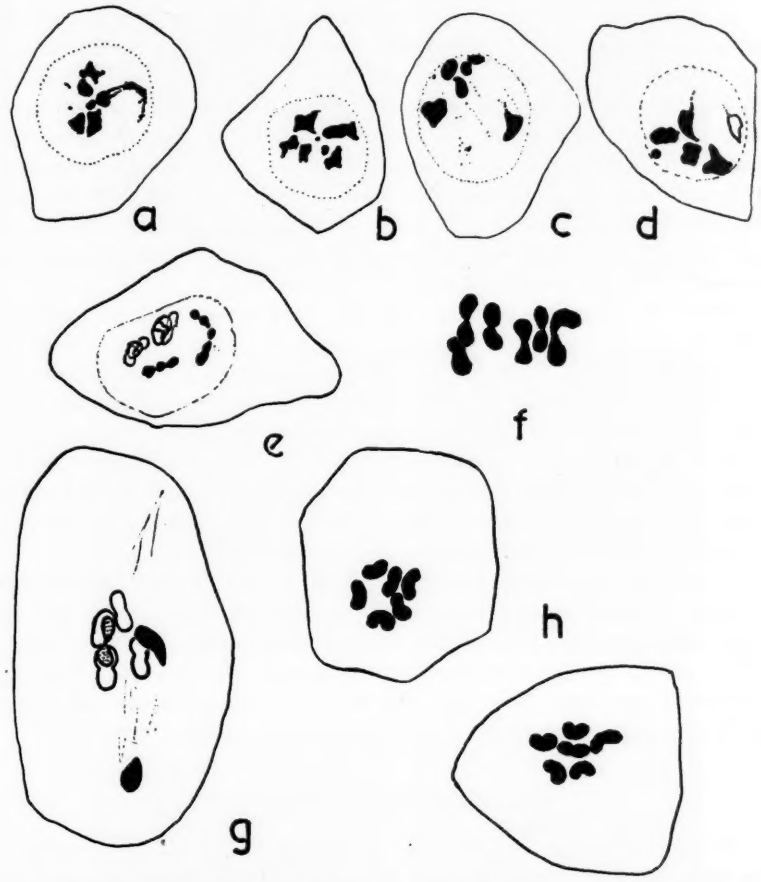

Fig. 4, a—h. *Phanaeus vindex.* (In all cases of this figure the neo-XY has been directed to the right.) — a and b. Neo-XY participating in the formation of the compound chromocentre. — c and d. Neo-XY opposite to the forming chromocentre; c, side view, d, polar view. — e. Late diakinesis. — f. First metaphase. — g. Neo-X and neo-Y separating precociously. — h. Second metaphase. — ×2480.

are terminal or nearly so. Never is there more than one chiasma per bivalent, and no univalents have been found, either. HAYDEN, whose description stops at late diakinesis, reports a high proportion of ring bivalents. Her findings are, consequently, not in accord with the present observations.

It is during the rapid recondensation phase that the longitudinal structure of the sex bivalent is best revealed. There is a short euchromatic segment at the end of both chromosomes. This segment is similar in both X and Y, and maintains the only regular contact, which may be a chiasma, between the sex chromosomes. The point of contact has been invariably terminal. Towards the other ends, thicker segments follow in both chromosomes. The neo-X is longer and ends with the old, thick X-remnant, already seen in the bouquet. In many cases the neo-Y shows a thin hair with a tiny knot at its tip — presumably a vestige of a small nucleolus, formed during the diffuse stage.

While the autosome bivalents are still more or less grouped together, the neo-XY typically has a separate location in the nucleus. HAYDEN is also obviously referring to the sex bivalent when she states that one of the bivalents tends to separate earlier from the group (p. 283). The neo-XY is inclined to form a pseudo-ring, owing to the frequent association of the free end of the neo-Y with the old-X remnant (Fig. 2). The centromere location of the sex chromosomes could not be limited with complete certainty, although a position much closer to the free ends than to the chiasma-forming ends is obvious.

After complete condensation, the autosome bivalents are dumb-bell shaped in the metaphase I (Figs. 4 f—g, 5 d). The methaphase plate is somewhat irregular, resembling that of *Onthophagus* species (VIRKKI 1951, 1954). The sex bivalent tends to separate sligthly earlier than the autosome bivalents. The sex chromosomes can be identified in the plates of the metaphase II, as well (Figs. 4 h, 5 e—f).

II. DORCUS PARALLELOPIPEDUS L.

For the family Lucanidae only one chromosome count is recorded in the literature. TOSHIOKA and YAMAMOTO (1937) have reported 19 chromosomes (XO) in *Psalidoremus inclinatus*.

Several clear late spermatogonial plates have shown the chromosome number of *Dorcus parallelopipedus* to be 2n = 18. Owing to the shortened chromosomes these late metaphases have afforded little further information regarding the chromosome structure, such as the length relation of the arms. Metacentrics seem to be prevalent, however (Figs. 6 a, 7 a). The sex chromosomes remain unknown.

Leptotene nuclei are diffusely stained and contain only very little autosomal heterochromatin, probably situated at the centric regions, and appearing as small granules. The sex bivalent appears at one side

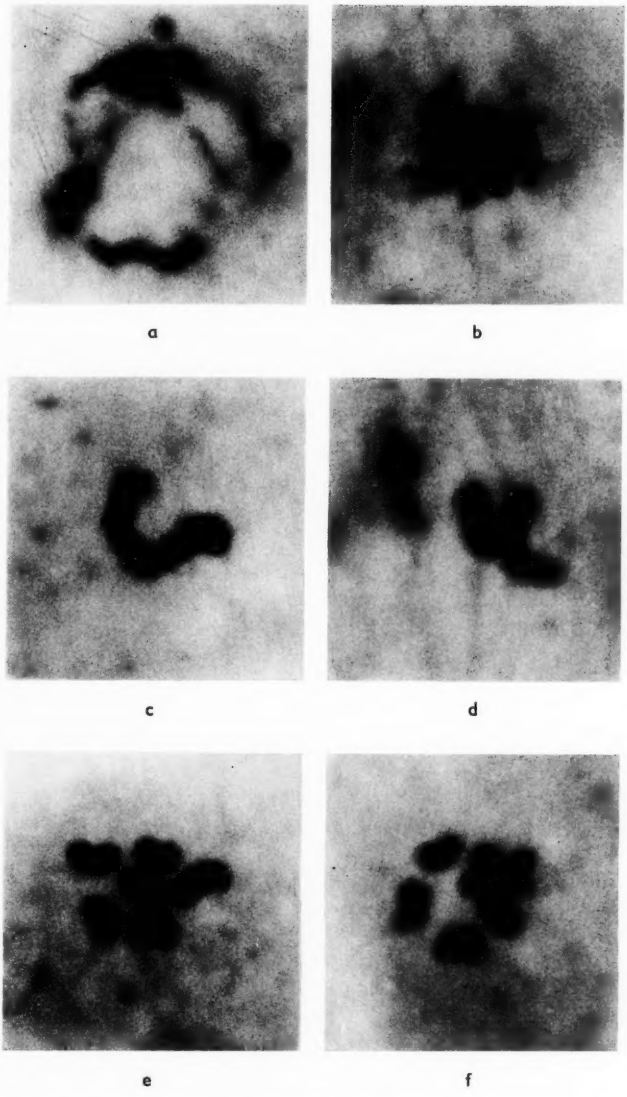

Fig. 5, a—f. *Phangeus vindex.* — a. Diplotene. Formation of the compound chromocentre ("karyosphere"), side view. Neo-XY opposite to autosome bivalents. — b. As in a, but polar view and neo-XY taking part in the formation of the chromocentre (Comp. Fig. 4, a and b). — c. Neo-XY in the first prometaphase. — d. Neo-XY in the first metaphase. — e and f. Second metaphase (same plates as in Fig. 4 h). Hematoxylin. — ×3650.

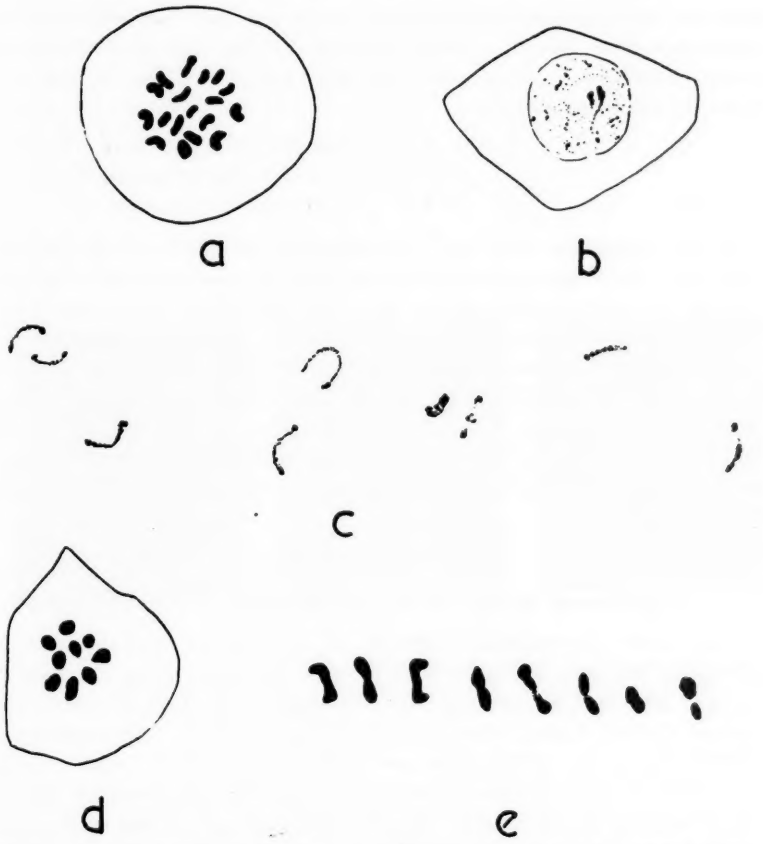

Fig. 6, a—e. *Dorcus parallelopipedus.* — a. Spermatogonial plate, 2n=18. — b. Leptotene, showing the bipartite structure of the sex bivalent and euchromatic parts. — c. Diplotene, all bivalents. Neo-XY at right. — d. First metaphase. — e. Side view of the bivalents at first metaphase. Neo-XY at right. — ×2480.

of each nucleus, but it is small, and very often bipartite (Fig. 6 b). A wide light area surrounds it. Both sex chromosomes seem to contain a euchromatic "tail", penetrating deep into the nucleus among the autosomal material.

Owing to the smallness of the cells and the lack of squashed material the development of single tetrads is hard to follow even in the late stages of prophase. Slight differences in the condensation of the

maternal and paternal chromosomes of the bivalents are recognizable sometimes. One chiasma, mostly located distally even in the earliest stages observable, is commonest, but rings are formed, too, as appears from the following sample:

0	1	2	3	Number of nuclei with 0, 1, and 2 ring bivalents, in
47	12	3	0	a sample of 62 nuclei.

In the metaphase I the sex chromosomes are easily recognized by their size. The shape of the bivalent suggests an association by chiasma. Among the autosomal bivalents there are two which sometimes look heteromorphous at the first sight, but this is due to a torsion of the metacentric chromosomes of the bivalent, which causes the free arms to lie in different radial planes. No heterokinesis or other irregularities have been observed in this or later stages. The main characteristics of the spermatogenesis of *Dorcus parallelopipedus* seem to me very normal, showing no unusual heteropycnosis or spermatocyte growth phenomena.

III. DISCUSSION

1. Evolutionary changes of the chromosomes in Scarabaeoidea

The basic chromosome number of the family Scarabaeidae is obviously the same as the basic number of the whole group Coleoptera, namely 2n=20. This has been found in about 82 % of all the scarabaeid genera studied (VIRKKI 1954). A typical representative of the scarabaeid chromosomes is metacentric; occasional acrocentrics may occur, especially in the geotrupins. The sex chromosome mechanism is almost invariably the Xy (Y smaller than X). The mode of pairing of X and y is probably always by the formation of a "parachute" (SMITH 1953), although this may easily remain undetected, especially if a sectioning technique has been used. Sex chromomes could not be distinguished from autosomes in *Phanaeus* species (according to HAYDEN 1925), or in *Geotrupes stercorarius* L. (VIRKKI 1951). In cetonines and trichiines the y chromosomes are very small (also in the Nearctic *Trichiotinus assimilis* KBY, VIRKKI unpubl.).

It is scarcely appropriate to speak of uniformity of the chromosome number and morphology in this large family, when only about 0.3 % of its species have been studied cytologically. The fact remains, however, that the present state of our knowledge provides very little information about the structural rearrangements of the chromosomes in

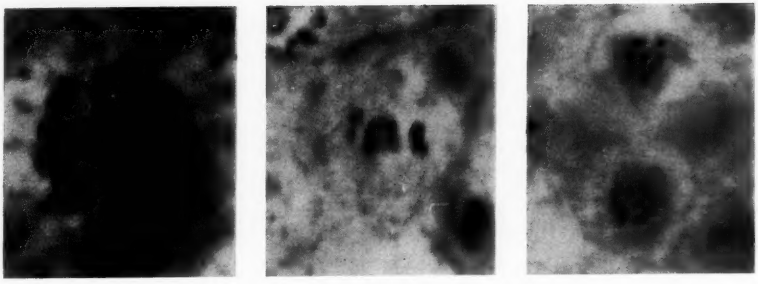

Fig. 7, a—c. *Dorcus parallelopipedus.* — a. Spermatogonial plate. — b. First metaphase, neo-XY at left. — c. Second metaphase. Neo-Y in the upper plate, neo-X in the lower. Hematoxylin. — a, ×4680; b—c, ×2130.

the Scarabaeidae. Only three points of divergence have appeared in the picture as yet:

Firstly, in the geotrupins 2n=22. This holds in Palearctic (VIRKKI 1951, 1954), as well as in Nearctic (*G. balyi* JEK., SMITH 1950; *G. splendidus* FAB., VIRKKI unpubl.) species. No link between this and the basic number has appeared. The first attempt to explain this difference would be by means of a fragmentation of two metacentrics to form four new (and two extra) chromosomes. In view of the presence of acrocentrics in the geotrupins this seems to be a sufficient working hypothesis, although, as pointed by WHITE (1954, 1957), such a fragmentation can scarcely be a simple process. The chromosome number is not the only characteristic which separates the geotrupins from the other scarabaeids, and even from other coprophages (CROWSON 1955, VIRKKI 1954 p. 27, and 1957). It seems probable that the geotrupins have long developed along a line of their own.

Secondly, in the Rutelinae and Dynastinae, the number 2n=18 is known to exist. Only one dynastine, *Oryctes nasicornis* L., has been studied (VIRKKI 1951).[1] The reduced number is perhaps due to an autosome-X fusion, in other words to neo-XY formation; but either the old X was very small, or further structural changes have taken place since the fusion, for the size of the sex chromosomes does not differ much. Great differences in the autosome sizes may, on the other hand, signify that other types of fusion have occurred.

In the rutelines, the tribe Anomalini contains both numbers: the basic 20 (*Blitopertha campestris* LATR., VIRKKI 1954) as well as a new number, 18 (in *Popillia japonica* NEWN., *Anomala corpulenta* MOTS., and *A. rufocuprea* MOTS., YOSIDA 1949). Further work in this section of scarabaeid systematics would presumably reveal relatively recent structural rearrangements.

Thirdly, a series of rearrangements has taken place in that group of coprophages which is also characterized by a remarkable growth of the spermatocytes during the diffuse diplotene, by large nurse cells, and by the more or less prolonged shape of the testis follicle (cf. VIRKKI 1957). In *Caccobius Schreberi* L. and *Oniticellus fulvus* GOEZE, only heteromorphous autosome bivalents have been found (VIRKKI 1954), in *Phanaeus*, the low diploid number 12.

Since HAYDEN's treatise (1925), the genus *Phanaeus* has been an enigma in the cytological picture of the Scarabaeidae. Now it appears

[1] PROWAZEK's (1902) earlier determination, 2n=12, is presumably erroneous.

from the present study that at least the last step of the decrease in chromosome number has taken place by means of an X-autosome fusion. This has brought the number from 2n=14 (presumably XO) down to 2n=12, neo-XY. What has been the mechanism of the decrease from 2n=20 to 2n=14 cannot be decided from the chromosomes, but it is possible that neo-XY formation alone has been repeatedly involved. In *Phanaeus vindex*, at least, no signs of interautosomal rearrangements can be ascertained.

Among the species previously studied by me, perhaps two *Copris*, *C. lunaris* L., VIRKKI 1954, and *C. tullius* OLIV., unpubl., come taxonomically closest to *Phanaeus*. These species have 2n=20, Xy_p, most of the chromosomes clearly metacentrics. I am, however, in possession of slides made of a Neotropical coprine[1], and it has 2n=14! Consequently, the coprines, especially the New World species, seem a very promising group for future studies.

In the formation of the "karyosphere" the neo-Y of *Phanaeus vindex* shows a slightly lesser degree of condensation in its free arm than the corresponding segment of the autosomal part of the neo-X. Later on, the distal free end of the neo-Y is positively heteropycnotic, tending to association with the old-X remnant. In general, the differentiation between the two former autosomes is not much, which suggests a relatively recent rearrangement. The segment lost by the autosome in the process of fusion is fairly long.

The other species studied here, *Dorcus parallelopipedus*, does not afford much data for discussion. The following three findings lead me to the conclusion that a neo-XY is involved: 1) the parachute form of the sex bivalent characteristic of beetles (SMITH 1950, 1953), is lacking, 2) both X and Y seem to have recognizable euchromatic segments (and probably a chiasmal association in the meiosis), 3) the only cytologically known relative, the lucanid *Psalidoremus inclinatus* (TOSHIOKA & YAMAMOTO 1937) has 19 chromosomes in the male, and an XO-system.

Here the heterochromatinization of the neo-Y has proceeded farther than in the case of *Phanaeus*. Obviously, we have in *Dorcus* a long established neo-XY, which is already near to the XY-system (or Xy, as SMITH (opp. cc.) puts it in order to emphasize the size difference).

[1] This case will be published in detail as soon as I have had the specimens identified. The beetles were collected by me in 1956 on the outskirts of the city of Tehuantepec, Southern Mexico. — *Addendum at correction:* The species has been recently identified by. Dr. O. L. CARTWRIGHT, Smithsonian Institution, Wash.; it is *Copris incertus* SAY.

2. Heterochromatin and structural rearrangements of chromosomes

Since the experimental work of DUBININ (1936) with *Drosophila*, the importance of the heterochromatin as a mediating factor in structural rearrangements has become obvious. Its significance is said to lie in its poverty in major genes, which allows loss of large chromosome segments without strong genetic effects. Similarly, when the chromosome number is decreased by neo-XY formation the heterochromatin plays a role, since it renders the whole y chromosome dispensible as well as large parts of the X. In the case of *Phanaeus vindex* the distally located autosomal heterochromatin has undoubtedly made possible a considerable loss of autosomal material in the process of translocation.

The other product of the autosome-X translocation, the centromere of the X carrying adjacent heterochromatic segments and the autosomal translocate, is generally doomed to rejection from the caryotype within the course of a few generations. In a Spanish *Oryctes nasicornis* L. I have encountered small, poor stainable accessory chromosomes, which could well be of such origin (VIRKKI 1954). *Epicometis hirta* PODA from Spain also comprises individuals with similar accessories, but the chromosome number is the basic 20, and Xy. These accessories have probably arisen by duplication of the small y chromosome, which they closely resemble in size and appearance. As their centromeres are, at least sometimes, functional, they must be taken into account as potential "points of anchorage" for future translocations. Similar but more degenerated accessory chromosomes have also been encountered in some other cetonines and trichiines with tiny y chromosomes (cf. VIRKKI 1954 a, p. 34, footnote).

The possibility that the heterochromatin plays a more active role in structural rearrangements also exists, but is merely speculative as yet. I mean the association of the heterochromites to form more or less persistent chromocentres, as frequently seen in many different organisms — the latest case in *Phanaeus vindex* here. The very intimate contact between heterologous chromosomes in chromocentres of the prophase or other phases suitable for chromosome fragmentation may well be an advantageous situation for the occurrence of heterosomal translocations. Since most of such compound chromocentres are formed by the procentric heterochromatin, this factor would cause whole-arm changes in the first instance. This factor is well represented in many scarabaeid suborders (VIRKKI 1951, 1953, 1954 a).

3. Notes on the development of the chromosome tetrads

In some scarabaeid species with a diffuse diplotene, the diakinetic reappearance of euchromatin is such a rapid process that it is hard to follow in detail from the preparations, owing to gaps between the various stages found. In *Phanaeus vindex* a few cysts of these rapid early stages have been found, however. HAYDEN obviously did not see them in her preparations, for she describes the bivalents as appearing from the "karyosphere" in the form of thick and well stained bodies.

When the euchromatic arms of a bivalent become visible, they are of approximately the same length as before the diffuse stage, but much thinner. The homologous chromosomes contact typically at two points only: at the heteropycnotic centric region, and at the only chiasma, which is invariably distally or terminally located. Under these circumstances the centromeric repulsion, which, according to DARLINGTON (1937), is the chief force of repulsion between homologues, has not contributed to the withdrawal of the euchromatic arms from another. Consequently, repulsive factors independent of the mutual repulsion of centromeres must exist.

Whether such an arm repulsion is sufficient to terminalize the chiasmata cannot be decided with certainty. It is true that even in the earliest recognizable tetrad stages only distal or even terminal chiasmata have been observed, but, on the other hand, distal localization is of common occurrence among scarabs, and even among some other groups of Coleoptera (cf. SMITH & MAXWELL 1953). According to observations on an elaterid beetle, *Agriotes mancus* SAY (VIRKKI 1958), the movement of the chiasmata is very slow during the earlier stages of bivalent condensation. Thus, a strict localization seems inevitable in *Phanaeus vindex*. The numerous cases with a terminal contact may have been caused, of course, by terminalization of a subterminal chiasma. In the neo-XY, however, the association of the sex chromosomes has always appeared to be a terminal one, from the earliest observable pachy-diplotene stages.

The opening out of the bivalents follows shortly after the breakdown of the procentric association, and the bivalents soon assume the usual late diakinetic appearance. The whole course of development somewhat resembles the case in *Blabera fusca* (SUOMALAINEN 1946), where paired chromosomes stay parallel until a sudden opening reveals a distally located chiasma. In *Phanaeus*, however, the factor keeping the chromosomes together is localized in nature and easily defineable as an association of the procentric heterochromatin.

Distal location of the chiasma is also prevalent in *Dorcus parallelopipedus*. Whereas the distal localization is accompanied by a positive and seemingly absolute interference of chiasma formation in *Phanaeus vindex*, *Dorcus* shows a frequent ring formation. This kind of difference cannot be estimated with any certainty from two individuals, for frequency and location of chiasmata so often differ even in individuals of one and the same species.

Aknowledgements. — Financial support for this study has been received from the Alfred Kordelin foundation and from the Finnish State Board of Science.

SUMMARY

Reinvestigation of the spermatogenesis revealed a typical neo-XY sex chromosome mechanism in *Phanaeus vindex* MACL., a Nearctic coprophagous scarab. The low chromosome number, 2n=12, and nonspecific prophasic associations reported by the former investigator (HAYDEN 1925) are confirmed here. The associations are due to procentric and distal heterochromasy of the autosomes, and to the sex bivalent. They persist through the diffuse diplotene until diakinesis, and cause a slight anomaly in the development of the late diplotene tetrads.

The lucanid studied here, *Dorcus parallelopipedus* L., likewise has a neo-XY, and 2n=18 in the male sex.

Both species (only one individual of each studied) show distal localization of chiasmata; in *Phanaeus*, the localization is strict, and a positive chiasma interference extends over the centromere.

Other evolutionary changes of the karyotype of the scarabs are briefly discussed on pages 489—490. The following new chromosome number determinations have also been mentioned in this connexion: *Geotrupes splendidus* FAB., 2n=22; *Copris tullius* OLIV., 2n=20, Xy_p; *Copris incertus* SAY, 2n=14.

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IMMUNO-ELECTROPHORETIC ANALYSES OF ALLOPOLYPLOID RYEWHEAT AND ITS PARENTAL SPECIES

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ANALYSES of specificity on the level of the macromolecules is basic to an understanding of the specification of the cells, the process which must ultimately determine the differentiation and variation of living organisms. The serological approach to studies of the structure of the macromolecules has proved useful for both plant and animal material. This paper describes an immuno-electrophoretic analysis concerning the specificity of the seed proteins of wheat and of rye and their hybrid. The study has been made in connection with experiments concerning the cytogenetic properties of the ryewheat (*Triticale*, 2n=56) which are being carried on at this institute (*cf.* MÜNTZING, 1956, 1957).

As early as 1901 KOWARSKI investigated the immunological properties of soluble substances in wheat and rye seeds and demonstrated antigenic similarities between these two species (KOWARSKI, 1901). Later MORITZ (1933) made a comparative study of the antigenic specificity of the seeds of ryewheat and the two parental species by means of anaphylactic reactions. He concluded from his results that some of the seed antigens in wheat and rye are identical while others are specific for the respective species and that the antigen composition of ryewheat includes the specific antigens of wheat and rye as well as the antigens they have in common. Both KOWARSKI and MORITZ used unfractionated extracts of the seeds for the serologic reactions and could therefore only make rough estimations of the similarities and differences between the antigen mixtures.

In the present investigation the immuno-chemical analyses have been carried out according to the immuno-electrophoretic technique described by GRABAR and WILLIAMS (1955). This technique permits a direct comparison between the immuno-chemical properties of electrophoretic fractions in antigen mixtures from different sources. By the

application of this method further information as to the macromolecular composition of the ryewheat and its parental species has been obtained.

I. MATERIAL

1. Plant material

The ryewheat line, the seed antigens of which have been analysed, is derived from a cross made in 1946 between a Swedish winter form of hexaploid wheat called Östgöta 01440 and an inbred line of Steel rye (Stålråg). This line had been inbred for about 25 years at the time when the cross was made. The allopolyploid hybrid is denoted Triticale O, and has been found to be a primary type with good vigour and fertility. It is now a part of the breeding material at the Institute. During the intervening years the rye line has been preserved by selfing.

2. Extracts

The macromolecules which were analysed were extracted from dry whole seeds. The seeds were ground into a fine powder which was suspended in 0.85 per cent Na Cl with the aid of a Turmix mixer. The ryewheat powder and the wheat powder were suspended in 1.5 times their weight of solvent and the rye powder in 2 times its weight of solvent. It was necessary to make the rye extract a little more diluted than the two others on account of the viscosus consistency of the rye extracts which make them difficult to filter. The suspensions were vigorously stirred by mechanical means for three hours at room temperature and were then placed in a refrigerator at $+4°C$ over night. The following day the suspensions were centrifuged and the supernatant fluid was sterilized by filtration through asbestos. Merthiolate was added in the proportion 1: 10000.

The solutions were not stable and became turbid after a few hours at room temperature. They had also a tendency to assume a dark colour but this was delayed by storing the extracts at $-20°C$. The flocculation, however, was unavoidable. When the extracts were used for immunization the flocculated material was stirred up and the whole suspension was injected, but when the extracts were used for the analytical reactions they were filtered through asbestos and the clear filtrate was used after adjusting the protein concentration. For the determination of the protein concentration trichloroacetic acid was added to the clear filtrates to a final concentration of 5 per cent. The precipitates were

washed in 5 per cent trichloroacetic acid and their nitrogen contents determined by the Kjeldahl technique.

3. Antisera

Three kinds of antisera were produced, antiwheat serum, antirye serum and antiryewheat serum. For the production of antisera adult rabbits were used, two for each extract, and the strongest antiserum was selected for the analyses. A volume of 1.5 ml. of extract was injected subcutaneously 3 times a week during 6 weeks. 12 days after the last injection 50 ml. of blood was taken from the marginal vein of the ear. The blood was kept at room temperature for 5 hours and then placed in the refrigerator at +4°C over night. Next day the clear serum was removed and mixed with merthiolate in the proportion 1: 10000. The serum was stored at —20°C.

II. METHODS

Simple electrophoresis in agar gel. — Before the immuno-electrophoretic analyses were made a preliminary investigation was carried out on the extracts by simple electrophoresis in agar gel. The purpose of this analytical fractionation was to determine the electrophoretic behaviour of the most important protein fractions in the extracts and to make a simple classification of these. The technique of GRABAR and WILLIAMS was used.

A 1.5 per cent agar gel in a 0.05-m sodiumveronal-H Cl buffer solution, with the pH value 8.2 and containing merthiolate in the concentration 1: 10000 was poured over a glass plate of the dimensions 180× 130×1 mm. so that a homogeneous agar film, 2,5 mm. thick, was formed over the plate. In this agar film oval reservoirs were cut out, 14 mm. long and 2.5 mm. broad, into which the extracts were pipetted. The extracts were mixed with equal volumes of 1 per cent agar at +45°C, and 0.075 ml. of this mixture was transferred into the reservoirs by a micro-pipette. The protein concentration in the extracts before mixing was adjusted to 10 mg/ml. The voltage between the electrodes during the electrophoresis was 100 volts which corresponds to a potential fall of about 4 volts/cm. in the separation zone. The strength of the current per plate was about 55 mA. The time of the electrophoresis was 4 hours.

The connection between the agar film and the buffer solution in the electrode vessels consisted of strips of filter paper, 4 cm. wide and 13 cm. long. The electrode vessels contained 0.05-m sodiumveronal-H Cl buffer solution with a pH value of 8.2.

For the fixation and colouring of the proteins the instructions given by URIEL and GRABAR were followed (URIEL, GRABAR, 1956). After fractionation the plates were placed in 2 per cent acetic acid for 3 hours in order to fix the proteins. The agar was then dried at +37°C over night, covered with a filter paper. Next day the filter paper was removed and the dried agar was carefully washed under running tap water. The plates were then placed in a solution of amidoschwartz. After 5 hours in this solution the excess colour was removed by washing in an aqueous solution of 2 % acetic acid and 15 % glycerine, and the plates were again dried at +37°C. The electrophoretic pattern was recorded by an Elphor densitometer.

Immuno-electrophoresis. — The immuno-electrophoretic technique is a combination of electrophoresis in agar gel with the double-diffusion technique of OUCHTERLONY (OUCHTERLONY, 1948). First the constituents of the antigen extract are separated by electrophoresis as described above. Then troughs are cut in the agar parallel to the electrophoretic track and antiserum is poured into them. The separated antigens and the antibodies diffuse into the agar and when they meet in adequate proportions they form stabilized precipitates in the shape of arcs, which are identified by their position, curvature and extent.

In the present investigation the troughs were 150 mm. long and 4 mm. broad and the distance between the antigens reservoirs and the immune serum troughs 7 mm. which proved to be the most suitable distance. The reaction time for the precipitations was 7 days during which time the plates were kept in a moist chamber at room temperature. The reactions were interrupted by placing the plates for 3 days in 0.85 per cent Na Cl which removed the reactants. The agar was then dried at +37°C, covered with a filter paper as described above and the precipitates coloured with azocarmine (URIEL, GRABAR, 1956).

Analyses were carried out both with unabsorbed and with absorbed antisera. The protein concentration in the antigen solution was 10 mg. per ml. in the analyses with unabsorbed antiserum and 9 mg per ml. in the analyses with absorbed antiserum. In making the absorption 0.1 ml. of extract containing 10 mg. protein per ml. was added to 1 ml. antiserum. The mixture was kept at +4°C over night, was centrifuged next day, and the supernatant fluid was removed.

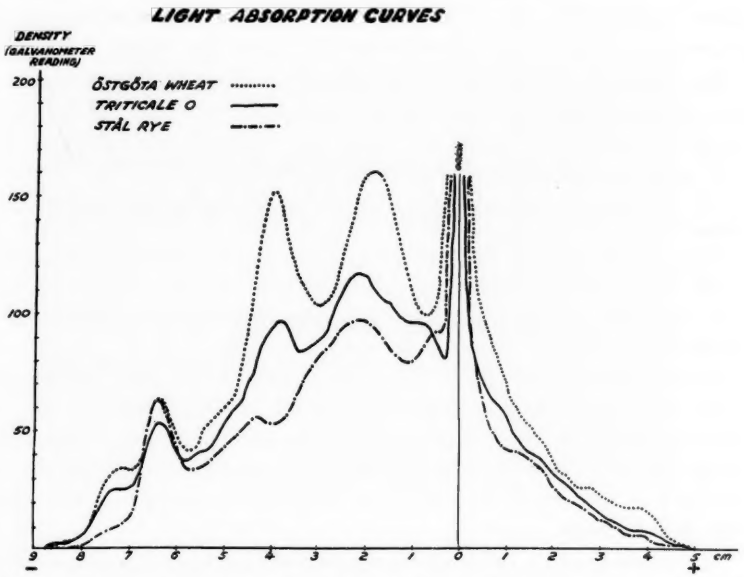

Fig. 1. Densitometer records of agar electrophoresis separation of saline extracts from seeds of wheat, rye, and ryewheat after staining with amidoschwartz.

III. RESULTS

Simple electrophoresis: The results of the simple electrophoresis are shown in Fig. 1 which is a graph of the densitometer records of the agar strips after colouring with amidoswartz. The classification of the fractions was made by measuring the distance between the antigen reservoirs and the absorption maxima. The analysis included only the fractions on the cathode side.

The protein mixture in the wheat extract was fractionated into 5 groups with the corresponding maxima at 1.8, 3.9, 5.4, 6.4, and 7.3 cm. distance. The proteins in the rye extract were fractionated into 4 groups at 2.1, 4.3, 6.4, and 7.3 cm. distance. Obviously two fractions in the two extracts (at 6.4 and 7.3 cm. distance) have the same electrophoretic mobility.

The protein mixture in the ryewheat extract gave 4 distinct maxima at 2.1, 3.9, 6.4, and 7.3 cm. distance and 2 less distinct bulges at about 4.3 and 5.4 cm. distance. *Thus the two fractions which were found to be common for the wheat and rye extracts are present also in the rye-*

wheat extract. The other fractions in the ryewheat extract correspond to fractions either in the wheat extract or in the rye extract.

Quantitatively the two fractions nearest to the antigen reservoirs are predominant in all three extracts. The area under the part of the curves which corresponds to these two fractions constitutes about 3/4 of the total area. According to the scale used the total area for the wheat curve is 797 planimeter units, for the rye curve 536 units, and for the rye-wheat curve 670 units. In spite of the fact that the same amount of protein was applied in all three cases there is a considerable difference in the total light absorption. The cause of this was not determined, but it is interesting to note that the ryewheat curve has a position intermediate between the wheat curve and the rye curve.

Immuno-electrophoresis: The immuno-electrophoretic experiments were arranged so that the homologous systems were always present in the middle between the two troughs. In the absorption experiments the antiserum had to react with the extract by which it was absorbed on one side of the trough and with the homologous extract on the other side of the trough.

The results of the immuno-electrophoretic analyses are collected in Figs. 2—8. The letter *ö* indicates wheat extract, *s* rye extract, and *t* rye-wheat extract.

The protein fractions, which were identified by the absorption readings after colouring with amidoschwartz, can be traced by the precipitation arcs which they formed. One band only, situated at the extreme end of the electrophoretic track in the homologous systems in figures 2, 6, and 8, has no relation to the electrophoretic pattern. Therefore the corresponding antigen present in the wheat and ryewhat extracts is probably not a protein. The quantitatively predominating fractions nearest to the antigen reservoirs formed severel overlapping bands, the individuality of which could not be distinguished because the antigen concentration in this part of the electrophoretic spectrum was too high in relation to the concentration of antibodies. The fractions situated farther away from the reservoirs, on the other hand, have formed bands which can be defined.

Fig. 2 illustrates the reactions by the unabsorbed antiwheat serum and Fig. 3 the reactions by the absorbed antiwheat serum. In the homologous system in Fig. 2 one of the precipitation bands formed a wavy-line with two maxima at a distance of 7.3 and 5.4 cm. from the reservoirs. The corresponding protein fractions at these distance (*cf.*

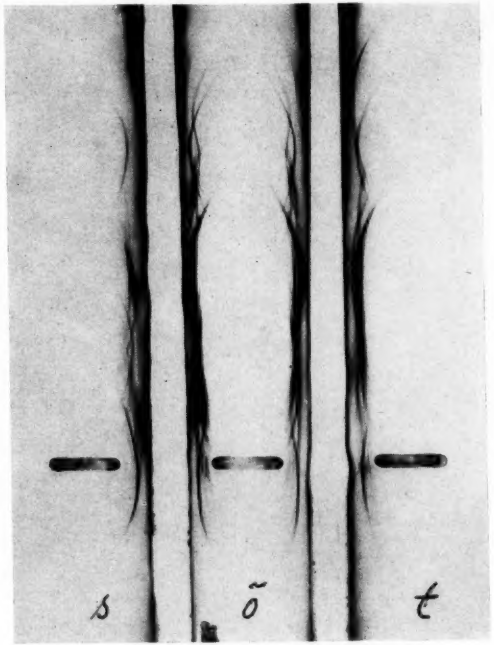

Fig. 2. Analysis by unabsorbed antiwheat serum.

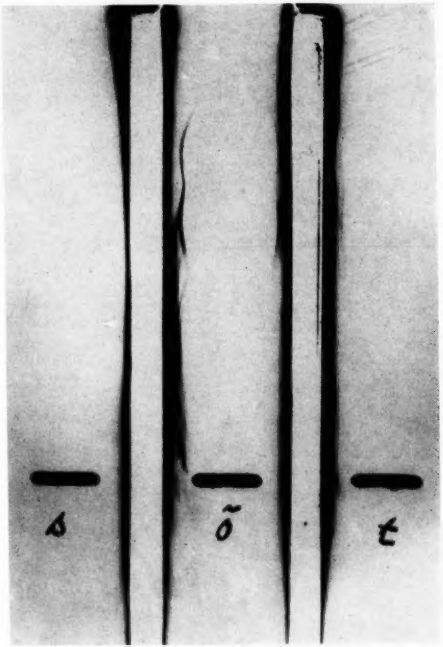

Fig. 3. Analysis by absorbed antiwheat serum.

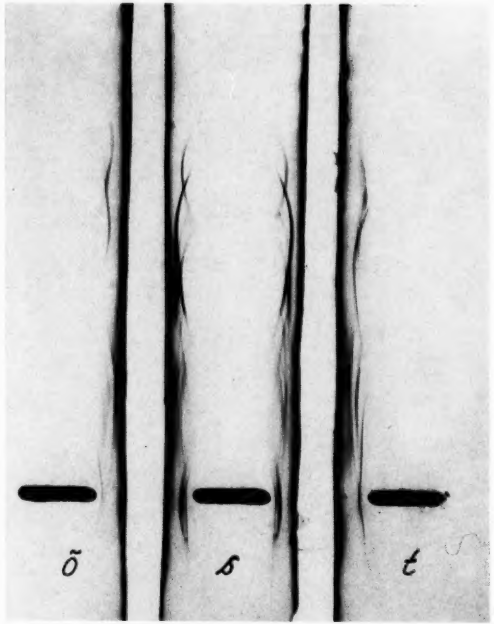

Fig. 4. Analysis by unabsorbed antirye serum.

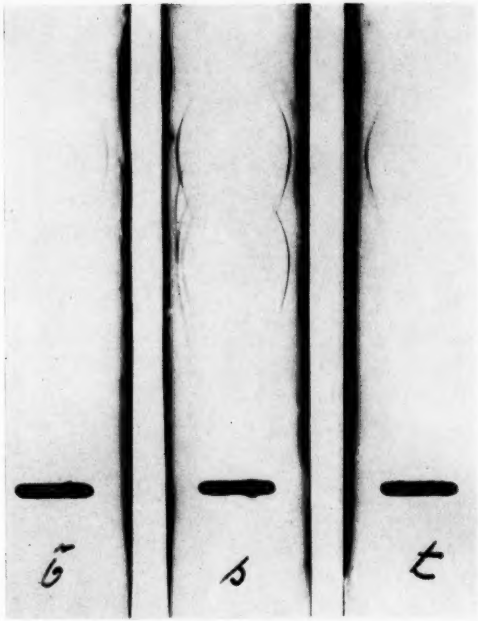

Fig. 5. Analysis by absorbed antirye serum.

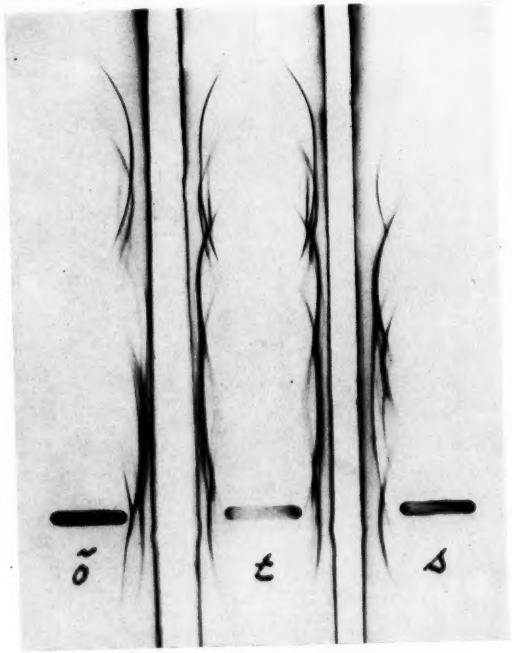

Fig. 6. Analysis by unabsorbed antiryewheat serum.

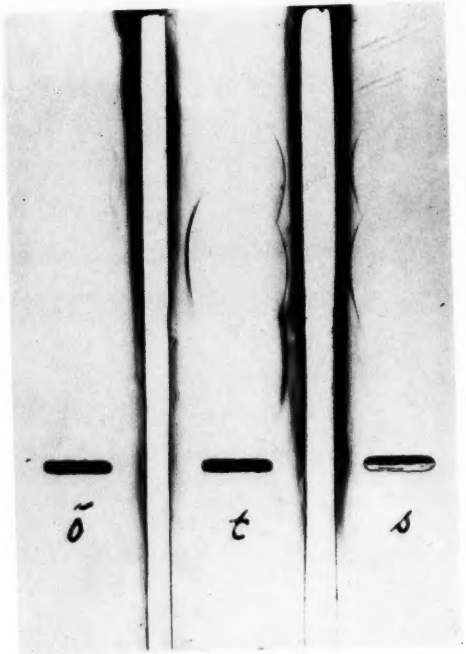

Fig. 7. Analysis by absorbed antiryewheat serum.

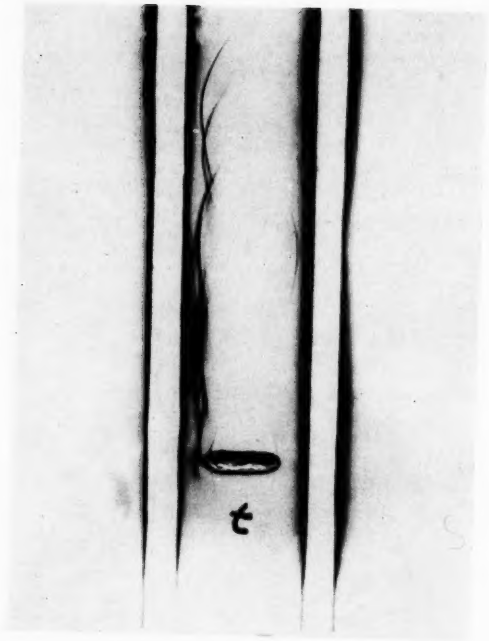

Fig. 8. Analysis by antiryewheat serum absorbed by wheat extract and rye extract.

Fig. 1) have different electrophoretic properties, but react with the same antibodies, giving rise to a wavy-line.

The reactions between the rye extract and the unabsorbed antiwheat serum formed one band at a distance of 6.4 cm. for which there is a direct correspondence in the homologous system, indicating immuno-electrophoretic identity between the antigens responsible for the precipitates. Besides this line several others were formed which are impossible to define in relation to the homologous arcs. The absorption by the rye extract showed that several proteins present in the wheat extract are lacking completely in the rye extract or occur there in small amounts only.

The bands formed by the reactions between the ryewheat extract and the unabsorbed antiwheat serum agreed in number, position and curvature with the bands in the homologous system. The absorption by the ryewheat extract was also complete (*cf.* Fig. 3) indicating that *all the antigens in the wheat extract which were capable of being defined immuno-electrophoretically were present also in the ryewheat extract.*

The results of the analyses with the unabsorbed anti-rye serum are demonstrated in Fig. 4. The wavy-line present in Fig. 2 is recognized in the two heterologous systems but not in the homologous system in Fig. 4. Thus, the corresponding antigens are present in all the three extracts. In the rye extract, however they are present only in amounts sufficient to form antibodies but insufficient to form visible precipitations. After absorption by the wheat extract the homologous system formed three lines and after absorption by the ryewheat extract one line all of which have no correspondence to the respective heterologous systems. *These results show that at least three proteins are peculiar to the rye extract as compared to the wheat extract, and that the rye extract contains one protein fraction which is lacking completely in the ryewheat extract or occuring there in small amounts only.*

Figs. 6—8 demonstrate the results of the analyses by anti-ryewheat serum. The reactions by unabsorbed antiserum are shown in Fig. 6 and by absorbed antiserum in Fig. 7. The left trough in Fig. 8 contained unabsorbed anti-ryewheat serum, and the right trough anti-ryewheat serum absorbed by wheat extract as well as by rye extract (1 ml. antiserum was mixed with 0.1 ml. of extract each of which contained 10 mg. protein per ml.). Some parts of the homologous system can be seen to correspond with the anti-ryewheat serum-rye extract system and other parts with the anti-ryewheat serum-wheat extract system. After the double absorption one very weak band was formed on the right

hand side in the middle field and this might be interpreted as indicating the presence of a substance peculiar to the hybrid. The rye extract, however, also formed a line with the same position (Fig. 6) and thus the line in Fig. 8 does not indicate a substance peculiar to the ryewheat extract. *No definite indications for the occurrence of specific "hybrid substances" were detected.*

IV. DISCUSSION

From the combined results of the electrophoretic and immuno-electrophoretic analyses it is evident that the wheat extract contains proteins which are lacking in the rye extract or occur there in small amounts only, and that the rye extract contains proteins which are lacking in the wheat extract or occur there in small amounts only. It is also evident that some protein fractions in the two extracts agree in their physico-chemical and immuno-chemical properties. KOWARSKI's *and* MORITZ's *statements of serologic similarities between wheat and rye have been confirmed, and it has been possible to identify some of the identical antigens by their electrophoretic mobility.* As regards the protein composition of the ryewheat extract, the combined results indicate that *this extract contains all the proteins identified in the wheat extract and all the proteins identified in the rye extract with one exception.*

The specific wheat proteins as well as the specific rye proteins apparently preserve their individuality even when they are synthesized in cytoplasm controlled by a nucleus containing the wheat genomes as well as the rye genome. Perhaps the fact that not all the rye specificities could be detected in the ryewheat extract indicates that the synthesis of some of the specific rye proteins can not occur in wheat cytoplasm.

The formation of hybrid substances found in some species hybrids of birds (*cf.* IRWIN, 1951) was not detected in these experiments, and the protein composition of the ryewheat as far as examined was found to have originated by a more or less complete addition of the proteins of the parental species.

DANIELSSON (1949) investigated by ultra-centrifugation seed globulins of different species in the *Gramineae* and found the same sedimentation constant for certain globulins in so widely separated species as *e.g. Secale cereale*, *Triticum vulgare*, *Avena sativa*, and *Festuca rubra*. DANIELSSON's investigations, although not directly comparable to the

investigations reported here, indicate nevertheless that the structures now detected in *Subtribus Triticinae* might be found in species only distantly related to this group.

The comparative electrophoretic and immuno-electrophoretic analyses showed that the number of antigenic proteins is greatest in the ryewheat extract and smallest in the rye extract. In other words the biochemical complexity increases in proportion to the degree of polyploidy.

There are several instances of wider geographic distribution of allopolyploids as compared to their diploid relatives (*cf.* MÜNTZING, 1953). This increased ecological capacity of the allopolyploids might perhaps be related to the possession of extensive enzyme systems formed by additions of the enzymes of the parental species in the same way as the reserve proteins have been added.

It is also possible that the good baking quality of some types of ryewheat might be related to the complicated mixture of macromolecules present in its seeds.

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SUMMARY

(1) Immuno-electrophoretic identity has been established between some soluble seed proteins from wheat and rye.

(2) All proteins immuno-electrophoretically identified in the wheat could be traced in the ryewheat.

(3) Indications have been found that ryewheat is unable to produce some of the proteins specific for rye.

(4) Specific ryewheat proteins could not be found.

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THE BEHAVIOR OF ACCESSORY CHROMOSOMES IN TETRAPLOID RYE[1]

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I. INTRODUCTION

ACCESSORY chromosomes have been extensively studied in diploid *Secale cereale* (MÜNTZING, 1945, 1946, 1949, and 1954). Studies at meiosis show that they may behave like chromosomes of the normal complement except the two groups never pair. When only one accessory is present, it behaves like a univalent and two may pair to form a bivalent although pairing may be reduced. Three or more accessories are capable of forming multivalents but are usually rare (HÅKANSSON, 1957). At A I lagging accessory univalents may divide and go to the poles (MÜNTZING and PRAKKEN, 1941). They may be excluded at A II and occur in the tetrads as micronuclei. At the first pollen anaphase the accessories usually show non-disjunction with the two chromosomes remaining attached at a special segment and appearing like a meiotic bivalent. These two chromosomes are usually included in the generative nucleus. This phenomenon has been confirmed by studying the progenies of crosses involving the same or varying numbers of accessory chromosomes. In these cases accessories in the progenies increase and these frequencies congregate about the even chromosome numbers. If only one accessory chromosome is present, pollen mitosis proceeds normally. With two accessories some disturbances in the divisions are apparent, and with three there is a complete upset of the division (HÅKANSSON, 1948, 1957).

It is interesting to see if the accessory chromosomes will behave in

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the same manner if the normal chromosome number is doubled. Thus, tetraploid plants with varying numbers of accessory chromosomes were obtained. The range of these chromosomes in the original tetraploid population was examined. Selected plants with two, four, five, six, seven, and eight accessory chromosomes were studied with respect to the behavior of the accessories in a tetraploid environment at meiosis, at mitosis of the first pollen division, and for transmission of the accessories to the progenies. Also, the first pollen anaphases were studied in the tetraploids to determine the extent of the disturbances in relation to the number of accessories.

II. MATERIAL AND METHODS

The tetraploid Transbaikal rye population was the C_3 generation after colchicine treatment of eleven plants. The progenies were isolated for one year to provide the seed used in this study. Each plant was labeled with the parental plant and spike numbers. The seedlings were grown in the greenhouse and root tips used to determine the chromosome numbers. Plants having accessory chromosomes were selected and clonally divided to provide more material.

Root tips for chromosome counts were fixed in Navashin-Karpechenko fixative. Sections were cut at 14 μ and stained with crystal violet. The material used for meiotic studies and for first pollen grain divisions were fixed in 4 parts absolute alcohol: 1 part glacial acetic acid and stored in a deep freeze. Meiosis was studied from acetocarmine slides which were made permanent by the dry-ice technique of CONGER and FAIRCHILD (1953) and mounted in Canada Balsam. The pollen mother cells were either in about the same stage of division or showed a wide range. This variation allowed several stages to be studied from the same slide thus eliminating some variation between anthers. Chromosome drawings were made with a camera lucida. The accessory chromosomes are shown in black and the normal complement is in outline. Metaphase I configurations were scored only for accessory chromosome configurations and the number of univalents in the normal complement. Cells in which the configuration of the accessory chromosomes could not be determined were discarded. However, records were kept of the number of cells in each category to see if there were large discrepancies associated with an increase of accessory chromosomes. In anaphase I and II the number of lagging chromosomes was tabulated. Many A I cells had divided laggards. Each partner was

counted as one-half a chromosome in tabulating so that the results could be compared with the number of metaphase univalents. Both plates of the A II cells were summed. At interphase and in the tetrads the number of micronuclei were also counted.

The first mitotic pollen grain divisions, which could easily be seen through the cell walls, were studied from temporary slides. Metaphase and anaphase plates were studied from the same slides. Only anaphase cells which had all the chromosomes clearly visible at both poles were selected for study since the accessories might separate and be hidden among the normal chromosomes. The accessory chromosomes were recorded as undergoing non-disjunction or as separated. If only the short arms of the accessory chromosomes had separated, then they were scored as still showing non-disjunction. The frequency of abnormal cells was also tabulated. These cells included both minor and major disturbances.

Tetraploid plants with two accessory chromosomes were isolated in a pollination chamber to permit intercrossing. One of these plants had a small iso-chromosome so only the transmission of the standard type was followed. Chromosome numbers were determined from paraffin sections of the root tips. The same procedure was repeated for groups with four, six, and eight accessory chromosomes. Unfortunately, seed setting could not be investigated since not enough plants were available to obtain material for both cytological studies and intercrossing. In some cases half the spike was collected for cytological studies and the remainder left for crossing.

A field planted population of diploid rye was also studied. Determinations of the chromosome numbers were made from the first pollen divisions since it would have been too difficult to obtain root tips. If one accessory chromosome was found, several slides were studied to see if a second one might also be there. The first pollen division was studied as in the tetraploid plants.

The terminology used in this paper will refer to the extra chromosomes as accessory chromosomes (for an explanation; see MELANDER. 1950) and the remainder as the normal complement (normal chr.). The two main types seen in this study were the standard accessory chromosome (st. acc. chr.) and the small accessory iso-chromosome (iso-chr.). Associations between these two types were called hetero-pairing.

TABLE 1. *Range of accessory chromosomes in a C_3 population of 178 tetraploid rye plants.*

	Number of standard accessory chromosomes																
	0	0 or 1	1	1 or 2	2	2 or 3	3	4	5	5 or 6	6	7	8	9	10	11	12
Plants with only st. acc.	71	2	20	4	12	1	0	9	3	1	5	1	1	0	0	0	1
Plants with st. acc. and/or other small pieces	33	0	3	0	5	0	2	1	0	0	1	1	1	0	0	0	0

III. RESULTS

The root tips in C_3 population plants were analyzed to determine the frequency and types of accessory chromosomes (Table 1). Some plants had only the standard accessory types described by MÜNTZING (1954) and others had, in addition, many small pieces and iso-chromosomes (MÜNTZING, 1944; MÜNTZING and LIMA-DE-FARIA, 1949 and 1953). In several plants the preparations were not clear enough to determine the exact count; therefore, two alternatives are given. Most plants (104 or 58 %) were without accessory chromosomes. Very high numbers of accessory chromosomes occurred in both types of plants since each could tolerate as many as eight standard accessories and one plant even had twelve standard accessories.

1. Plants with two accessory chromosomes

After studying each stage, it appeared that there was a large amount of variability among the plants which, whenever it was possible to test, did not seem to appear within a plant. Thus, it seemed of more interest

TABLE 2. *Metaphase I standard accessory chromosome associations in 4n+2 st. acc. chr. plants.*

Plant no.	Types of st. acc. chr. associations		Total no. of cells	Cells with normal I's
	2 I	1 II		
40—1	71.4 %	28.5 %	84	8.3 %
40—2[1]	78.3	21.6	157	17.8
40—6	84.3	15.7	83	—
40—11	87.2	12.8	86	41.9
43—3[1]	81.4	18.6	167	—

[1] average of two slides.

TABLE 3. *Metaphase I associations of accessory chromosomes in 4n+2 st. acc. chr.+1 small iso-chr. plants.*

Plant no.	Types of acc. chr. associations			Total no. of cells	Cells with normal I's
	2 I + 1 sm. iso-chr.	1 II + 1 sm. iso-chr.	1 II (1 acc. chr. + iso-chr.) + 1 I		
41—4[1]	92.7 %	4.3 %	3.0 %	164	29.9 %
45—2[1]	98.1	0.6	1.2	161	13.0
45—10	98.2	1.8	0	113	38.0

[1] average of two slides.

to study the frequencies in each individual plant. First, the appearance of the various stages and the types of abnormalities are discussed for this group of plants.

Meiotic metaphase I stages were studied in five plants with two standard accessory chromosomes (Fig. 1 B) and in three which had in addition a small iso-chromosome (Fig. 1 C). As can be seen in Tables 2 and 3, most of the cells contained accessory chromosome univalents. Usually they were located on the periphery of the cell. Fig. 1 B also shows two normal univalents located at the edge of the plate. The accessory chromosome bivalents are usually orientated on the plate. Their shapes and those of the hetero-bivalents are like those illustrated by MÜNTZING (1951 b). In two plants where two slides were studied, agreement was found to be fairly close (78 % and 79 % for plant 40—2, and 85 % and 78 % for plant 43—3); therefore, the results were averaged. The five plants with only two standard accessory chromosomes averaged 80 % of the cells with accessory chromosome univalents. The plants which had, in addition, a small iso-chromosome showed a very low amount of conjugation (only 4 %, Table 3). Since it is possible that an overall reduction of conjugation was responsible, the pairing of normal chromosomes in plants without accessory chromosomes, and with

TABLE 4. *Per cent of PMC's in metaphase I showing the number of normal chr. I's in 4n plants with no and one acc. chr.*

	No. of normal I's				Total no. of cells
	0	1	2	3	
Plants with no acc. chr.	76 %	14 %	8 %	2 %	100
Plants with one acc. chr.	81	8	11	0	100

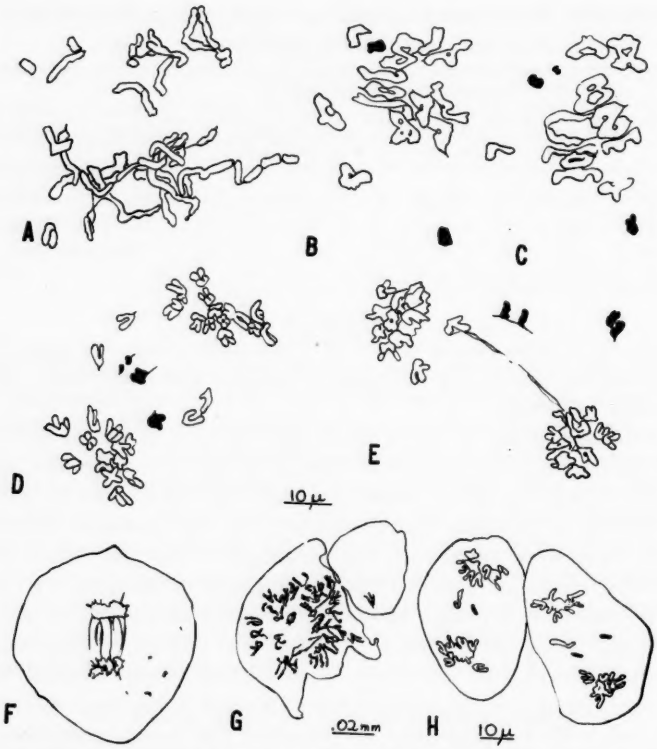

Fig. 1. Various meiotic stages in 4 n+2 standard accessory chromosome rye plants. — A. Questionable metaphase I cell in plant 40—2, sticky and fragmented. — B. Metaphase I (plant 43—3), 2 acc. chr. I+3 I+9 II+1 III?+1 IV. — C. M I in plant 45—2 which has in addition 1 small iso-chromosome, 6 II+4 IV+2 st. acc. chr. I+ 1 sm. iso-chr. I. — D. A I in plant 45—2 showing 2 lagging acc. chr. I's and 1 sm. iso-chr. in process of division plus 2 divided chr. of the normal set. — E. Plant 43—3 with 2 st. acc. chr. (1 dividing), ½ lagging normal chr. plus a normal chr. bridge. — F. An abnormal late telophase cell with about nine bridges in plant 40—2. — G. An interphase nucleus with a bud containing a small amount of chromatin — cytomixis ? — plant 45—2. — H. A II in plant 40—1 showing lagging chromosomes in each plate: Left, 1 st. acc. chr.+1 normal chr.; right, 2 st. acc. chr.+1 normal acc. chr. Acc. chr. in black. — Figs. A—E magnified as to the upper scale; F, and G to lower left; and H to lower right.

only one accessory chromosome was studied (Table 4). If the number of normal univalents are compared (Tables 2, 3, and 4), there does not appear to be an increase of normal univalents in the plants with the small iso-chromosome. Apparently, the small iso-chromosome decreased

TABLE 5. *Per cent of anaphase I PMC's showing lagging chromosomes in 4n+2 st. acc. chr. plants.*

Plant no.	No. of lagging st. acc. chr.					Total no. of cells	% cells with		
	0	1/2	1	1 1/2	2		bridges	lagging normal chr.	lagging iso-chr.
40—2[1]	34.0 %	1.9 %	15.5 %	5.8 %	42.7 %	103	1.9 %	36.5 %	0 %
40—6[1]	26.2	2.5	22.5	5.0	43.8	80	5.0	33.8	0
43—3[1]	25.6	0.6	24.4	3.8	45.6	160	2.5	40.6	0
45—2[2]	10.2	1.3	16.7	7.7	64.1	78	5.1	15.3	73.1

[1] average of two slides.

[2] one small iso-chr. also present.

the pairing of the standard accessory chromosome. One abnormal cell, probably in metaphase, is shown in Fig. 1 A. It had highly fragmented and sticky chromosomes.

The standard accessory chromosomes at anaphase I lagged in about three fourths of the cells, whereas the plant with the small iso-chromosome had even a higher number of laggards (Table 5). In some cases the accessory chromosomes had already divided or were in the process of division (Fig. 1 E). In a low per cent of the cells, one half of the chromosome might have reached the pole while the other half lagged. The small iso-chromosome behaved like the standard accessory chromosome (Fig. 1 D and Table 5). There were also as many as three lagging normal chromosomes which could divide at A I (Fig. 1 D). The per cent of normal laggards was higher in the plants without the small iso-chromosome. A few cells had normal chromosome bridges (Fig. 1 E and Table 5). An abnormal late telophase cell with nine bridges and many fragments is shown in Fig. 1 F.

The majority of the interphase cells were without micronuclei although

TABLE 6. *Per cent of micronuclei at interphase of 4n+2 st. acc. chr.*

Plant no.	Number of micronuclei					No. of cells
	0	1	2	3	4	
40—2	78.9 %	17.8 %	3.3 %			90
40—6	80.0	18.0	0.0	2.0		50
43—3[1]	60.0	28.0	9.1	2.3	0.6	175
45—2[2]	49.0	32.0	15.0	3.0	1.0	100

[1] average of two slides.

[2] one small iso-chr. also present.

as many as four were seen in a cell (Table 6). These could arise from two sources: lagging accessory chromosomes and lagging normal chromosomes. In spite of this difficulty, the number of micronuclei is lower than expected from the results of the A I data, provided that two lagging chromosomes do not form one micronucleus. Thus, the divided A I accessory chromosomes usually must move to the poles. Abnormal interphase cells were seen with many micronuclei and with the nuclear material dumbbell shaped. An abnormal cell was seen which contained a cytoplasmic bud with a small amount of chromatin (Fig. 1 G).

Anaphase II gave a similar picture to anaphase I, since many cells had lagging chromosomes. The two plates of a cell often showed the same number of laggards (70 %). Thus, in Table 7, only a few cells had just one lagging accessory chromosome. Fig. 1 H shows a cell where the number of accessory chromosome laggards was different in both plates and there was also one normal chromosome laggard. As many as four normal chromosome laggards were seen in a cell. A few cells with dot-like chromosomes were also observed. Also, the normal chromosomes might be stretched out between the poles. When the iso-chromosome was present, it lagged in about 60 % of the cells.

Abnormalities at the earlier meiotic stages were reflected in the tetrads as micronuclei. The micronuclei ranged up to ten in a plant (Table 8) and varied in size. Also there were no obvious modal classes. Two of the plants had a lower frequency of normal tetrads. A low percentage of abnormal cells were seen as diads or triads with varying numbers of micronuclei.

In the first pollen mitosis both metaphases and anaphases were studied. In spite of a wide variation at metaphase between the four plants (Table 9), most of the cells had either no or one accessory chro-

TABLE 7. *Per cent of anaphase II PMC's with lagging chromosomes in 4n+2 acc. chr. plants. Sum of laggards in both plates.*

Plant no.	No. of lagging acc. chr.					No. of cells	% cells with	
	0	1	2	3	4		lagging normal chr.	lagging iso-chr.
40—1	33.8 %	4.4 %	14.7 %	23.5 %	23.5 %	68	63.2 %	0.0 %
40—2	35.2	1.4	36.6	11.3	15.5	71	14.1	0.0
40—6	14.9	14.9	29.8	8.5	31.9	94	61.7	2.1 (?)
45—2[1]	11.1	9.5	22.2	38.1	19.0	63	22.2	60.3

[1] one small iso-chr. also present.

TABLE 8. *Per cent of tetrads with micronuclei and abnormalities in 4n+2 acc. chr. plants.*

Plant no.	No. of micronuclei											Abnormal cells	Total tetrads
	0	1	2	3	4	5	6	7	8	9	10		
40—1	27.8 %	9.6 %	20.0 %	18.9 %	14.4 %	4.4 %	2.6 %	0.7 %	0.0 %	0.4 %	0.4 %	0.7 %	270
40—2[1]	21.9	24.7	29.9	11.6	8.4	1.6	1.2					0.8	251
40—6[1]	6.4	10.7	18.7	21.1	25.1	8.4	4.0	3.3	1.0	0.3		1.0	299
45—2[2]	6.1	12.5	28.4	21.2	18.2	8.7	1.9	0.8	0.4	0.4		1.5	264

[1] some cells on slide still in process of division.

[2] one small iso-chr. also present.

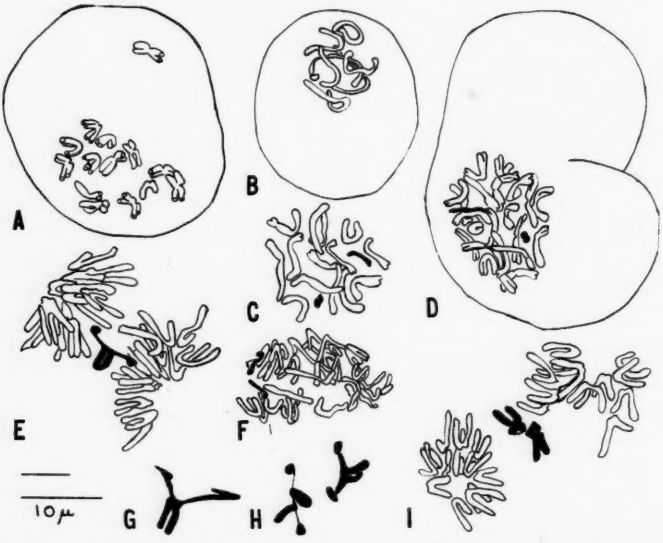

Fig. 2. First pollen grain mitosis in 4 n+2 standard accessory chromosome rye plants. — A. Plant 40—2 with 14 chromosomes, one chromosome located out by itself. — B. A cell in plant 45—2 with only seven chromosomes. — C. Metaphase plate in plant 45—2 with 14 chromosomes+1 st. acc. chr.+1 small piece. — D. A pollen metaphase containing 23 normal chromosomes+1 st. acc. chr.+probably 1 sm. iso-chr. (plant 45—2). — E. Anaphase with a 14—14 separation of normal chromosomes, 1 st. acc. chr. with the short arms separated (plant 40—2). — F. Anaphase in plant 45—2; 13—13 separation of the normal complement with 2 normal chromosomes parallel to the plate, 1 st. acc. chr. separated. — G. A st. acc. chr. showing separation of the short arms and the centromeres directed to the poles. The long arms remain parallel and are held together by the special segment (plant 40—6). — H. 2 st. acc. chr. in plant 45—2 similar to Fig. 2 G except the short arms have completely separated. — I. Normal anaphase in plant 40—2 with a 14—14 separation of the normal chromosomes, and 2 st. acc. chr. both showing non-disjunction. Acc. chr. in black. — Figs. A—D, F magnified as to upper scale, and Figs. E, G—I by lower scale.

mosome. A typical metaphase plate of the plant containing an iso-chromosome is shown in Fig. 2 C. It has fourteen normal chromosomes, a standard accessory chromosome, and probably a small iso-chromosome. In addition to this small iso-chromosome, a deleted accessory chromosome was seen in one plate. Only a few cells in these plants had an iso-chromosome. The normal chromosomes ranged from twelve to sixteen owing to irregular separation of the multivalents and elimination of the lagging univalents. A metaphase with thirteen chromosomes

TABLE 9. *Metaphase of first pollen mitosis in 4n+2 st. acc. chr. plants. Per cent of cells with acc. chr.*

Plant no.	No. of slides	No. of acc. chr.					Total no. of cells
		0	1	2	1+1 iso-chr.	1 small acc. chr.	
40—2	11	36.8 %	56.3 %	6.9 %			87
40—6	4	56.2	34.2	6.8	1.4 %	1.4 %	73
43—3	1	74.1	22.2	3.7			27
45—2[1]	3	46.3	49.2	1.5	3.0		67 + 2 haploids

[1] one small iso-chr. present.

grouped together and one isolated is shown in Fig. 2 A. In several cells, chromatin drops were present in the cytoplasm. Two haploid cells were seen in plant 45—2, one with seven normal chromosomes (Fig. 2 B) and the other with nine. A tetraploid cell with twenty-three normal chromosomes, one standard accessory chromosome and probably one small iso-chromosome, was also observed in the same plant (Fig. 2 D). MÜNTZING (1949) reported a correlation on a slide between the per cent of binucleate pollen and the number of accessory chromosomes in pollen metaphase or anaphase. Therefore, about 350 pollen grains per slide were scored as to whether they were binucleate. For example, one slide had 69 per cent of the pollen binucleate and had 24 per cent of the anaphase cells without accessory chromosomes. Another slide with about the same number of anaphase cells and with 60 per cent binucleate pollen had 74 per cent of the cells without accessories. No relationship was visible between these two factors; therefore, the data were summed.

In pollen anaphase, typical non-disjunction of the accessory chromosome occurs (see Fig. 2 I for a cell with two chromosomes undergoing non-disjunction). Zero to two accessory chromosomes were seen in the pollen (Table 10). One half of the cells lacked accessory chromosomes; this fact agrees quite well with the metaphase counts. Also, in metaphase about 5 % of the cells had two accessory chromosomes and at anaphase 3 % of the cells. No iso-chromosomes were seen. In about 17 % of the cells that had accessory chromosomes, non-disjunction had failed and the accessory chromosomes had separated (Fig. 2 F) or were in the process of separation (Figs. 2 E, G, and H). The centromeres apparently were able to function normally since the short arms went to the poles. However, it is the "special segment" which holds the two

TABLE 10. *Per cent of cells showing non-disjunction of accessory chromosomes at anaphase of first pollen mitosis in 4n+2 standard accessory chr. plants.*

Plant no.	No. of slides	No. of st. acc. chr.					Total no. of cells
		0	1		2		
			non-disjunction	separated	non-disjunction	1 acc. separated	
40—2	4	66.7 %	25.9 %	0 %	7.4 %		27
40—6	5	44.1	54.3	0	1.7		59
43—3	1	50.0	40.6	6.2	3.1		32
45—2[1]	3	50.0	29.6	18.0	1.3	1.3 %	78+1 haploid

[1] one small iso-chr. present.

long arms together and parallel (MÜNTZING, 1948). In some cases this region may also be pulled out and attenuated (see Figs. 2 H left, 6 J, and 7 C).

Abnormalities were seen in a few pollen anaphase cells. These were slightly higher for cells with one accessory chromosome than those with none (Table 32). With two accessory chromosomes, this number increased, but there were too few cells to draw any definite conclusions. The mildest abnormalities consisted of a slight scattering or lagging of the chromosomes as they moved to the poles. In other cases, one end of a normal chromosome might remain attached while the other was near the pole. Some chromosomes remained parallel to the plate (Fig. 2 F) after the other chromosomes had started to go to the poles. Usually these were minor deviations and would not prevent the cells from forming functional pollen. Only about 6 % of all the pollen grains appeared to be abnormal. The abnormal cells did not appear to be correlated with the chromosome number of the normal complement.

The transmission of the standard accessory chromosomes was studied in the root tips of the progeny of intercrossed plants. The number of standard accessory chromosomes ranged from one to four (Table 11) and averaged 0.81 accessory chromosomes. Forty-nine per cent of the plants were without accessory chromosomes at pollen metaphase and 50 % at anaphase. This result is higher than expected from intercrossed plants of this constitution. Possibly the pollen grains without accessory chromosomes might have had a selective advantage over those with accessory chromosomes or the viability of zygotes with accessory chromosomes might have been reduced. Unfortunately, counts could not be

TABLE 11. *Transmission of standard accessory chromosomes to the progenies from intercrossed plants with 4n+2 st. acc. chr. in their root tips.*

Female parent	No. of st. acc. chr. in progeny						Total no. of plants
	0	1	1 or 2	2	3	4	
40—2	7	6	0	2	1		16
40—6	3	4	0	2	1		10
43—3	4	3	1	0	1	1	10
45—2[1]	9	8					17
%	43.4	39.6	1.9	7.5	5.7	1.9	53

[1] 1 small iso-chr. present in root tips.

made of the amount of seed set. Germination of the seedlings was good, so probably elimination did not occur here. The next highest class of plants had only one accessory chromosome which could not be predicted from metaphase and anaphase counts. The accessory chromosomes might disjoin at late anaphase resulting in an increase of plants with one accessory chromosome. Another possibility would be the elimination of an accessory chromosome at a later division. The low number of plants with three and four accessory chromosomes prevents confirmation of this possibility. There did not appear to be a bias in selecting the cells which could be analyzed because tabulation of eliminated cells showed that many of them contained at least one accessory chromosome in the process of non-disjunction. About 28 % of the cells in the root tips of intercrossed progenies had an iso-chromosome or small dot. Thus, the transmission of the small iso-chromosome was fairly good.

The frequency of the accessory chromosomes at the various stages will now be discussed for each plant.

Plant 40—2

At metaphase I, 78 % of the accessory chromosomes were univalents and 18 % of the normal chromosomes (Table 2). The number of cells without lagging chromosomes at A I (Table 5) was 34 %. The per cent of these cells without accessory chromosome laggards is higher than expected from the number of metaphase cells with univalents. This plant has the smallest number of accessory laggards of the four plants and a much higher number of normal chromosome laggards than two of the plants. At interphase, 79 % of the cells lacked micronuclei, a higher percentage than two of the plants (Table 6). In A II (Table 7), the number of cells without laggards was 35 % which is almost

the same as in A I, and the normal chromosome laggards were only 14 %, the lowest of the plants. This plant was one of two that had more tetrads without micronuclei (22 %, Table 8) which probably resulted from the higher number of cells at A II without lagging accessory chromosomes.

In metaphase and anaphase of the pollen divisions, cells without accessory chromosomes were 37 % and 67 %, respectively (Tables 9 and 10). The first value agrees closely with A I and A II, and is the lowest of the four plants. However, the anaphase results were hard to explain and are high in contrast to those of the other plants. The intercrossed plants gave an almost equal number of progeny with zero and one accessory chromosomes (Table 11).

Plant 40—6

This plant had at M I less conjugation (84 % of the cells with I's) than the preceding plant (Table 2) and also fewer A I cells without lagging accessory chromosomes (26 %, Table 5). The normal chromosome laggards were also slightly lower. In interphase (Table 6) about the same number of cells were without micronuclei (80 %) as in the preceding case. The A II cells with accessory laggards were increased from plants 40—1 and 40—2 (Table 7) since only 15 % of the cells were without. Many more normal chromosomes were lagging (62 %) than in two other plants. The increase of laggards in the previous stages is reflected by a large number of tetrads with micronuclei (only 6 % without, Table 8) and by a wider range of micronuclei (up to nine in a cell). At metaphase of the pollen division (Table 9), 56 % of the cells lacked accessory chromosomes and at anaphase, 44 % (Table 10).

Plant 43—3

The number of M I cells with only I's (Table 2) was intermediate between the first two plants (81 %). At A I, this plant was like 40—6 (Table 5) since 26 % of the cells showed accessory chromosome laggards. However, the number of lagging normal chromosomes was higher (41 %). Forty per cent of the interphase cells had micronuclei (Table 6). Metaphase of the first pollen division (Table 9) had 74 % of the cells without accessory chromosomes, whereas at anaphase only 50 % were without (Table 10).

Plant 45—2

This plant is the only one of the four given intensive study that had a small iso-chromosome. At M I (Table 3) the pairing of the accessory chromosome was greatly reduced (98 %) compared with the other plants and the normal chromosome I's had not increased (13 %). The low metaphase pairing is reflected by 90 % of the A I cells having lagging accessory chromosomes (Table 5). The normal chromosome laggards were fewer (15 %) agreeing with the metaphase data. Since there were more laggards, there were also more cells with micronuclei (51 %) and a wider range of micronuclei (Table 6). Again at A II, only 11 % of the cells were without accessory laggards (Table 7) and the normal chromosome laggards were again low (22 %). Most tetrads (94 %) had micronuclei which could range up to nine per cell (Table 8). More cells (54 %) had accessory chromosomes at pollen metaphase than in two of the preceding

TABLE 12. *Association of accessory chromosomes in metaphase I PMC's of 4n+4 st. acc. chr. rye plants.*

Plant no.	Types of acc. chr. associations					Total no. of cells	Cells with normal I's
	4 I	2 I+1 II	2 II	1 III+1 I	1 IV		
41—3[1]	82.2 %	16.8 %	0.5 %	0 %	0.5 %	208	21.1 %
51—1	58.8	33.3	2.0	0	5.9	51	5.9
40—3	78.8[2]	19.2[2]	0	1.9[2]	0	52	50.0
40—10	18.4	40.8	30.6	8.2	2.0	49	34.7

[1] sum of two slides.
[2] 1 I missing.

plants (Table 9). This value agrees with the 50 % without accessory chromosomes at anaphase (Table 10).

Summarizing this group with two standard accessory chromosomes, one might conclude that the general averages for each stage are rather unreliable. Yet, within a plant, definite patterns in the transmission of the accessories are indicated. Between plants, relative patterns could also be seen.

2. Plants with four accessory chromosomes

An even more extreme variation between the four plants in this group was noted in M I than in the preceding group (Table 12). For example, plant 40—10 had much more pairing and more multivalents than the other three plants. Also, between the plants the number of normal univalents varied widely. Since there are again such large divergences, it is probably more meaningful to discuss the various stages on a plant basis.

Plant 41—3

This plant at metaphase showed very low conjugation (Table 12) which was confined mostly to the formation of a single bivalent. Normal univalents were found in 21 % of the cells which is about what was found in the previous group. In anaphase I (Table 13), few cells were without lagging accessory chromosomes (17 %) and with lagging normal chromosomes (9 %). Sometimes the accessory chromosomes were also seen located over the poles. Three per cent of the cells had bridges. Fifty-two per cent of the interphase cells lacked micronuclei (Table 14) which is considerably higher than expected from A I. Occasionally, remnants could be seen of the A I bridges. Laggards at A II (Table 15) were even more numerous and could range up to as many as seven in the two plates. Only two per cent of the cells had bridges. The frequency of lagging normal chromosomes was about the same as in A I (18 %) and a

TABLE 13. *Anaphase I. Per cent of PMC's with lagging acc. chr. in 4n+4 acc. chr. plants.*

Plant no.	Number of lagging acc. chr.									No. of cells	Cells with bridges	Cells with normal laggards
	0	1/2	1	1 1/2	2	2 1/2	3	3 1/2	4			
41—3[1]	16.9 %	7.6 %	31.3 %	6.8 %	16.1 %	0.8 %	13.6 %	2.5 %	4.2 %	118	3.3 %	9.3 %
51—1	36.5	11.5	26.9	7.7	9.6	0	7.7			52	9.6	25.0
40—3[1]												
2 polar	53.7	3.7	22.2	0	7.4	0	13.0			54	1.8	31.5
3 polar	34.0	4.0	22.0	2.0	26.0	0	12.0			50	4.0	72.0
40—10												
2 polar	37.7	6.6	23.0	3.3	21.3	0	3.3	0	4.9	61	8.2	21.3
3 polar	60.0	0	0	0	40.0					5	0	80.0

[1] sum of two slides.

TABLE 14. *Interphase in PMC's of 4n+4 acc. chr. plants showing the per cent of micronuclei.*

Plant no.	No. of micronuclei					No. of cells
	0	1	2	3	4	
41—3[1]	51.7 %	36.6 %	9.0 %	2.1 %	0.7 %	145
51—1	54.0	34.0	12.0			50
40—3						
2 polar	92.1	6.3	1.6			63
3 polar	68.2	24.2	6.1	1.5		66
40—10						
2 polar	86.0	14.0				57
3 polar	66.7	0	33.3			3

[1] sum of two slides.

few dots were also observed. Tetrads without micronuclei were low (12 %, Table 16) which agrees quite well with the A II results. Two cases of triads with micronuclei were seen.

In the first pollen metaphase, as many as four accessory chromosomes were seen per cell and there were from thirteen to fifteen normal chromosomes. There were a few cells without accessory chromosomes (Table 17), about an equal number of cells with one and two, and a few with three and four. These values agree closely with the anaphase accessory chromosome frequencies (Table 18). A pollen grain with four accessory chromosomes had sticky, pulled out, and diffuse chromosomes (Fig. 4 C) which were twisted in relation to each other. A maximum of ten accessory chromosomes were transmitted to the progeny (Table 19). These high values might be expected from the anaphase results. However, no accumulation of accessory chromosomes was seen in the even classes. Plants with only one accessory chromosome were the most frequent.

Plant 51—1

About twice as many cells had a bivalent at M I as the preceding plant (41 %, Table 12) and a few quadrivalents were also present. Only a few normal chromosomes (6 %) were univalents. Correspondingly, more cells were without laggards at A I (37 %) which agrees closely with metaphase expectations (Table 13). Lagging normal chromosomes were present in one fourth of the cells and 10 % of the cells had bridges. Many laggards must have moved to the poles since 54 % of the interphase cells were without micronuclei (Table 14). A few bridges could still be seen. At A II as many as eight lagging accessory chromosomes were seen in the two plates and only 16 % of the cells were without micronuclei (Table 15). Like plant 41—3, about one half of the interphase cells were without micronuclei. The frequency of normal laggards was only 14 % and bridges, 6 %. Tetrads could have as many as

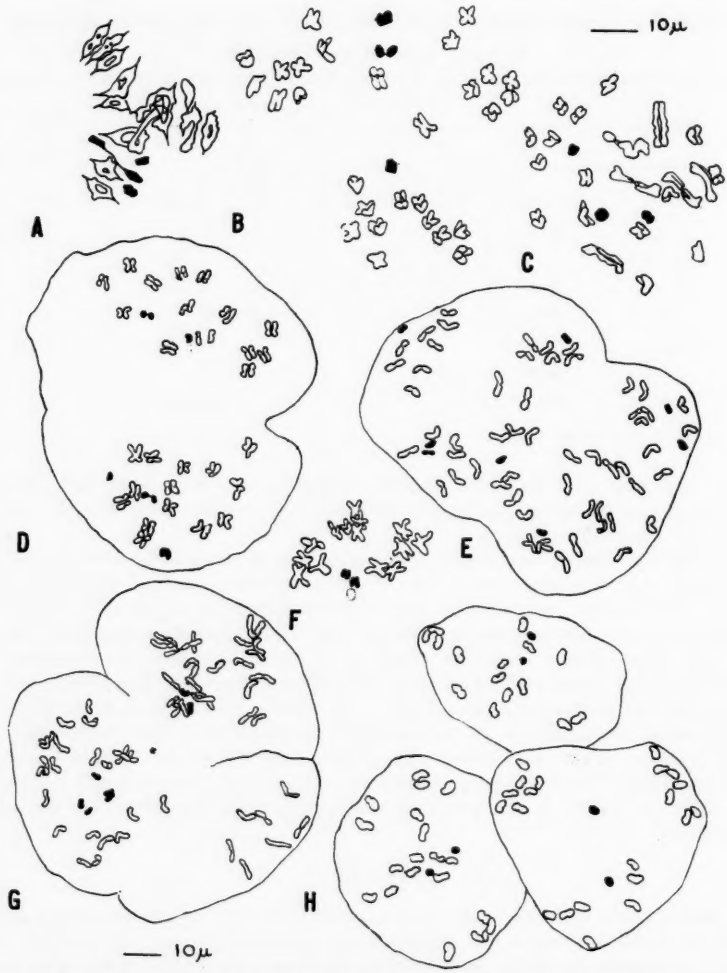

Fig. 3. Meiosis in 4 n+4 standard accessory chromosome rye plants. — A. Abnormal M I in plant 40—10. Y-shaped plate present. 3 IV+8 II+2 acc. chr. I+1 acc. chr. II. Note IV directed towards three different poles. — B. An anaphase with three poles in plant 40—3. 2 normal and 3 st. acc. chr. lagging (only 3 st. acc. chr. present). — C. An A I cell in plant 40—3 with misdivision of the normal chromosomes and abnormal distribution of the chromosomes. — D. M II with (bottom) 14 normal chr.+5 st. acc. chr. and (top) 14 normal chr.+3 st. acc. chr. Chromosomes shortened as in colchicine treated cells (plant 40—10). — E. Abnormal A II cell in plant 40—10. 58 normal chr.+8 st. acc. chr. Chromosomes contracted, scattered, or in several small groups. — F. A M II plate in plant 40—10 with the nucleolus still present (14 nor-

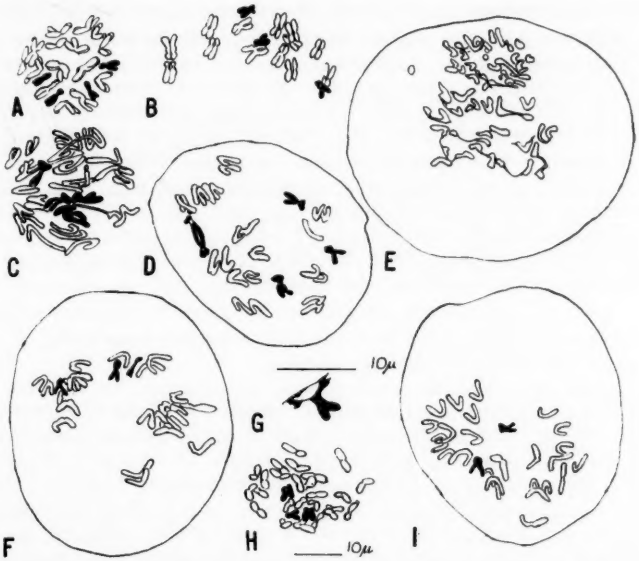

Fig. 4. Tetraploid rye plants with 4 standard accessory chromosomes in the root tips. First pollen grain mitosis. — A. Metaphase with 4 st. acc. chr.+15 normal chr., chromosomes contracted, plant 51—1. — B. A contracted metaphase cell in plant 51—1. 13 normal chr.+3 st. acc. chr. — C. Disturbed pollen anaphase in plant 41—3. 14—14 distribution of the normal chr. with 4 st. acc. chr. undergoing non-disjunction. Several chr. pulled out. — D. Tripolar anaphase in plant 51—1 with a 9, 8, and 7 distribution of normal chromosomes. Three st. acc. chr. showing non-disjunction and an apparent dicentric acc. chr. — E. Cell with a much fragmented metaphase (?) stage (plant 40—10). — F. Anaphase with 3 st. acc. chr. undergoing non-disjunction (plant 51—1). Either a tripolar cell or else there are 3 normal+2 acc. chr. lagging, and 3 normal chr. at bottom are directed towards the bottom of the cell. — G. An acc. chr. undergoing non-disjunction in plant 40—10. Note 2 centromeres at each end. — H. 26 normal chr.+3 st. acc. chr. (plant 51—1). Very contracted chromosomes which have divided but show no poleward movement. — I. Either a unipolar cell or else two poles pressed into each other. 26 normal+2 acc. chr. plant 51—1. Acc. chr. in black. — Figs. A—F, H, I magnified by lower scale, and Fig. G by upper scale.

mal+2 st. acc. chr.). — G. An A II cell in plant 40—10. Left plate: 24 normal chr.+5 st. acc. chr. and a chromatin drop; lower right: 7 normal chr.; upper right: 25 normal+2 st. acc. chr., cell cleavage just starting. Chromosomes appear ragged. — H. Complete separation into 3 A II cells (plant 40—3). Top: 15 normal chr.+2 st. acc. chr.; left: 20 normal+2 st. acc. chr.; right: 21 normal+2 st. acc. chr. Note the tripolar spindle. Acc. chr. in black. — Figs. A—C magnified by upper scale, and D—H by lower scale.

TABLE 15. *Anaphase II. Per cent of cells with lagging chromosomes in 4n+4 acc. chr. plants. Sum of laggards in both plates.*

Plant. no.	No. of acc. chr. laggards									No. of cells	% of cells with		
	0	1	2	3	4	5	6	7	8		dots	lagging normal chr.	bridges
	%	%	%	%	%	%	%	%	%				
41-3	7.4	15.9	17.0	21.3	22.3	6.4	5.3	4.3		94	2.1	18.1	2.1
51-1	16	14	36	12	12	4	4	0	2	50	0	14	6

eight micronuclei although 10 % of the cells were free from them; this result corresponds well with A II data (Table 16).

The modal accessory chromosome class at metaphase of the first pollen mitosis was two accessory chromosomes (Table 17) which is higher than in plant 41—3. The classes with one and three accessory chromosomes (Fig. 4 B) were about equal and a few cells had four (Fig. 4 A). The zero classes in this plant and the preceding plant were both low. The sum of the classes with one and two accessory chromosomes are about the same in these two plants. The difference between plants 51—1 and 41—3 appears to be related to the greater amount of M I pairing in plant 51—1. Many of the pollen grains had extremely contracted metaphases (Figs. 4 A and B). However, it does not appear that the degree of contraction was directly associated with the number of accessory chromosomes since cells with one to four accessory chromosomes were contracted, whereas others with two and three were not.

The anaphase distribution (Table 18) is similar to metaphase. In some cells, accessory chromosomes had separated or else the short arms were in process of separation. One cell had three standard accessory chromosomes showing non-disjunction and one chromosome appeared to be a dicentric made up of two accessory chromosomes (Fig. 4 D). The division appears to be upset and is tripolar with a seven, eight, and nine distribution of the normal chromosomes. Another abnormal cell is shown in Fig. 4 F. This cell is either tripolar or else there are three normal plus two standard accessory chromosomes lag-

TABLE 16. *Per cent of tetrads with micronuclei in 4n+4 acc. chr. plants.*

Plant no.	No. of micronuclei										Abnormal cells	No. of cells
	0	1	2	3	4	5	6	7	8	9		
41—3[1]	11.5 %	11.0 %	21.6 %	23.4 %	17.0 %	8.2 %	4.1 %	0.9 %	1.4 %	0.9 %	0.9 %	218
51—1	10.3	15.2	24.0	22.5	14.2 %	8.3	3.4	1.0	1.0		0	204
40—10	24.6	9.8	19.2	13.8	11.3	10.8	6.9	2.5 %	1.0		0	203

[1] some divisions still present.

ging. There are also three normal chromosomes at the bottom of the cell which are directed away from the pole. An extremely contracted anaphase cell is shown in Fig. 4 H. The chromosomes had divided, yet no poleward movement was visible. Another type of cell which caused many difficulties is shown in Fig. 4 I. This cell could be interpreted as either a unipolar cell or else two poles that had been completely pressed into each other. Since all the chromosomes were on the same plane, the latter explanation does not seem as likely as the first. Also, note the normal chromosome at the bottom of the cell which was directed to the outside edge. Some chromosomes in other cells were parallel to each other in ski-like pairs. At late anaphase or early telophase, three to four groups of chromosomes were also seen. Transmission to the progeny ranged from zero to eight standard accessory chromosomes (Table 19). In a few cases a small dot or small iso-chromosome was also present.

Plant 40—3

Metaphase cells of this plant appeared to have one accessory chromosome missing although it was present in the root tips. Pairing was, however, as good as in plant 41—3 (Table 12) and a few III's were seen. One half the cells had normal chromosome univalents which was a much higher frequency than in the other three plants. At A I two types of divisions were seen. Some were normal bipolar types, whereas others had three poles (Table 13). An A I cell with three poles can be seen in Fig. 3 B. Only three accessory chromosomes were present and one appeared to be dividing. The two types of anaphases, bi- and tripolar, seemed to give different percentages of lagging accessory chromosomes although when there were three poles it was sometimes rather arbitrary to judge if a chromosome was lagging. Since the percentages of cells with one lagging chromosome were the same, the discrepancy occurs in those cells with two laggards. The number of bridges was low in both cases. However, lagging normal chromosomes were much higher in the tripolar types. When the tripolar types were discovered at A I, the M I plates were re-examined. Many cells had Y-shaped plates like in Fig. 3 A. This distribution of the normal chromosomes was probably an indication of tripolar A I spindles. Misdivision of the normal chromosomes (Fig. 3 C) was another abnormality in the tripolar cells. There was about the same difference between the two

TABLE 17. *Metaphase of first pollen mitosis in 4n+4 standard accessory chromosome plants. Per cent of cells with accessory chromosomes.*

Plant no.	No. of slides	No. of acc. chr.						Total no. of cells
		0	1	2	3	4	2+3 iso-chr.	
41—3	2	7.8 %	37.2 %	47.0 %	5.9 %	2.0 %		51
51—1	2	3.6	16.4	65.4	12.7	1.8		55
40—3	4	17.1	57.1	22.8	2.8			35
40—10	1	6.7	26.7	53.3	13.3			30
45—15	6	8.8	32.9	31.9	22.0	3.3	1.1 %	91

TABLE 18. *Anaphase of first pollen mitosis in 4n+4 standard accessory chromosome plants. Per cent of cells showing non-disjunction of acc. chromosomes.*

Plant no.	No. of slides	No. of accessory chromosomes									Total no. of cells
		0	1		2		3			4	
			non-disj.	separated	2 non-disj.	both separated	3 non-disj.	2 nondisj. 1 separated	1 nondisj. 2 separated	3 nondisj. 1 dicentric	all non-disj.	
41—3	2	10.3 %	27.6 %	6.9 %	44.8 %	0	6.8 %	0	0	0	3.4 %	29
51—1	3	15.3	7.7	3.8	42.3	3.8 %	19.2	0	3.8 %	3.8 %		26
40—3	4	30.8	11.5	19.2	34.5	3.8						26
40—10	3	0	30.0	16.7	39.9	0	10.0	3.3 %				30
45—15	6	21.2	44.2	5.8	21.2	0	7.7					52

types of cells at interphase (Table 14) as at A I. However, as was the case in the two previous plants, there was a large increase of interphase cells without micronuclei compared with A I cells without laggards. As many as three micronuclei were seen per cell. No counts were made at A II and in the tetrads since it was very hard to distinguish which cells belonged together because of the tripolar divisions. Fig. 3 H shows an anaphase composed of three separate cells. The chromosome distribution appeared to be rather irregular and there were many laggards although two accessory chromosomes were distributed to each cell. One cell (lower right) contained a tripolar spindle. Many "tetrads" were also composed of three cells and had many micronuclei.

The modal class at pollen metaphase was one accessory chromosome (Table 17) and up to three accessory chromosomes were seen. More cells were without accessory chromosomes in this plant than in the previous two. A few cells in this plant were also contracted and in one a normal chromosome was separated from the others. The distribution at anaphase was different from metaphase (Tables 17 and 18). In some anaphase cells the accessory chromosomes were in process of separation, and others had a large amount of stickiness. Also, a few cells had extremely contracted chromosomes and poles twisted in relation to each other. In the progeny the accessory chromosomes again ranged up to eight (Table 19). The modal class (one acc. chr.) contained almost half of the progeny.

Plant 40—10

Pairing at M I among the accessory chromosomes was much higher than in the other three plants (Table 12). Most of the cells had either one or two bivalents and ten per cent of the cells had multivalents. In contrast, quite a high number of normal chromosomes were univalents. In this plant as in the previous one, Y-shaped spindles were present (Fig. 3 A). Note the quadrivalent directly above the accessory chromosomes has its centromeres directed towards three different poles. Also, one cell with a higher and one with a lower count than expected were observed. Anaphase I cells again showed types with two and three poles (Table 13) although there were only a few tripolar cells compared with plant 40—3. The tripolar types had fewer accessory chromosome laggards than the bipolar but had more normal laggards than the bipolar. This result is about what was obtained in the previous plant. A few cells had bridges and one cell contained three. At interphase only three cells were tripolar and the rest were bipolar (Table 14). In the latter case, about the same per cent of the cells were without micronuclei as in the preceding plant. Anaphase II in this plant as well as in the previous one was too difficult to score because of the tripolar divisions. Many other abnormalities also seemed to be present in these A II cells. Fig. 3 D shows a M II cell which had chromosomes that appeared shortened as if they had been colchicine treated (compare with Figs. 3 E and F). It had three standard accessory chromosomes in one plate and five standard accessory chromosomes in the other. In other cells up to seven normal chromosomes were seen lagging. The nucleolus was sometimes still visible at M II (Fig. 3 F). A large amount of stickiness was sometimes present at both metaphase II and anaphase II. The A II cells had

TABLE 19. *Transmission of accessory chromosomes to the progeny of intercrossed plants with 4n+4 st. acc. chr. in their root tips.*

Female parent	No. of st. acc. chr. in progeny											Total no. of plants
	0	1	2	3	4	5	6	7	8	9	10	
41—3	2	6	1	2	3	0	0	0	3	0	1	18
51—1	1 + 3[1]	2 + 1[1]	2	1[1]	3	0	1	0	1			15
40—3	1	8	3	2	0	2	1	0	1			18
40—4	2	3	3 + 1[1]	2	3	0	1	0	2			17
%	13.2	29.4	14.7	10.3	13.2	2.9	4.4	0	10.3	0	1.5	68

[1] contains in addition 1 small dot or sm. iso-chr.

many different gradations in their degree of division into cells. The chromosomes were so scattered that it was hard to distinguish two plates (Fig. 3 E). In addition, many normal chromosomes were lagging and a tripolar spindle was in the upper half. This cell also appeared slightly contracted. The cell in Fig. 3 G was partly divided and had three chromosome groups of unequal size. The chromosomes also had a ragged appearance. Fig. 3 H shows a complete division into three cells and has already been described above. One fourth of the tetrads were without micronuclei which is much higher than in the other two plants (Table 16).

In the pollen metaphase (Table 17) only a few cells were without accessory chromosomes. Cells with one and two accessory chromosomes show the same general trend as the first two plants (41—3 and 51—1). Fig. 4 E shows a peculiar cell which was assumed to be in metaphase. The chromosomes were highly fragmented. All the anaphase cells had from one to three accessory chromosomes with one as the modal number (Table 18). A majority of the accessory chromosomes showed non-disjunction. Fig. 4 G shows one of these which appeared to have neo-centric activity. In a few cells the normal chromosomes separated at different rates and were at different distances from the poles. The progeny from this plant was not examined.

Plant 45—15

Only the pollen divisions from this plant were studied. The metaphase distributions showed almost equal numbers of one and two accessory chromosomes (Table 17). In contrast to the other plants, many cells had three accessories or even four. A few cells had ski-like pairs while others had one or two chromosomes on the opposite side of the cell from the other chromosomes. The normal chromosomes ranged from eleven to seventeen. Sometimes small dots resembling iso-chromosomes were seen, although one cell appeared to have three iso-chromosomes. Anaphases had many cells without accessory chromosomes or with only one in contrast to the other plants (Table 18).

In general, tetraploid plants with four standard accessory chromosomes showed extreme variability in M I pairing. Probably the degree of M I pairing in plants 41—3 and 51—1 is reflected by the number of A I cells without lagging accessory chromosomes. The other two plants were probably more variable because of the tripolar spindles. The per cent of cells in interphase without micronuclei again showed that many A I laggards probably reached the poles and also this per cent is related to the per cent of M I pairing. More laggards were present at A II than A I although the plant that had the most laggards at A I also had the most at A II. The micronuclei in the tetrads followed the patterns of the previous meiotic stages.

The average pollen metaphase values are 8, 33, 43, 13, and 2 % for zero to four accessory chromosomes. The corresponding series for anaphase is: 16, 37, 36, 10, and 0.6 %. Both series appear to agree quite well. About 12 % of the anaphase cells were abnormal. In the progeny of the intercrossed plants, the modal class was one accessory chromosome (Table 19). This low odd-numbered class predominating is contradictory to the high amount of non-disjunction seen at pollen anaphase. However, some mechanism for increase must be effective since the mean number of accessory chromosomes was twice as high in the progeny (2.80) than it was at pollen anaphase (1.42). It appears, therefore, that the same mechanism may be operating here as in the 4n+2 accessory chromosome plants. The even-numbered classes do not predominate except in those with many accessory chromosomes.

3. Plants with six standard accessory chromosomes

In the plants with six standard accessory chromosomes, a very wide range again existed between the individual plants although the general trends can be compared with the two preceding groups. No tabulations were made of the micronuclei in either interphase or tetrads since they did not appear to provide any additional information. The various stages of meiosis and mitosis are described for each plant in Tables 20 to 24.

Plant 49—3

About one fourth of the metaphase I cells did not show conjugation of the accessory chromosomes. Most of the other cells had either one or two bivalents but a few had three. Multivalents were present in only 8 % of the cells. Normal univalents were relatively high. In anaphase I some cells contained as many as five lagging accessory chromosomes, although most had only one or two and many had none. About the same per cent of normal chromosomes

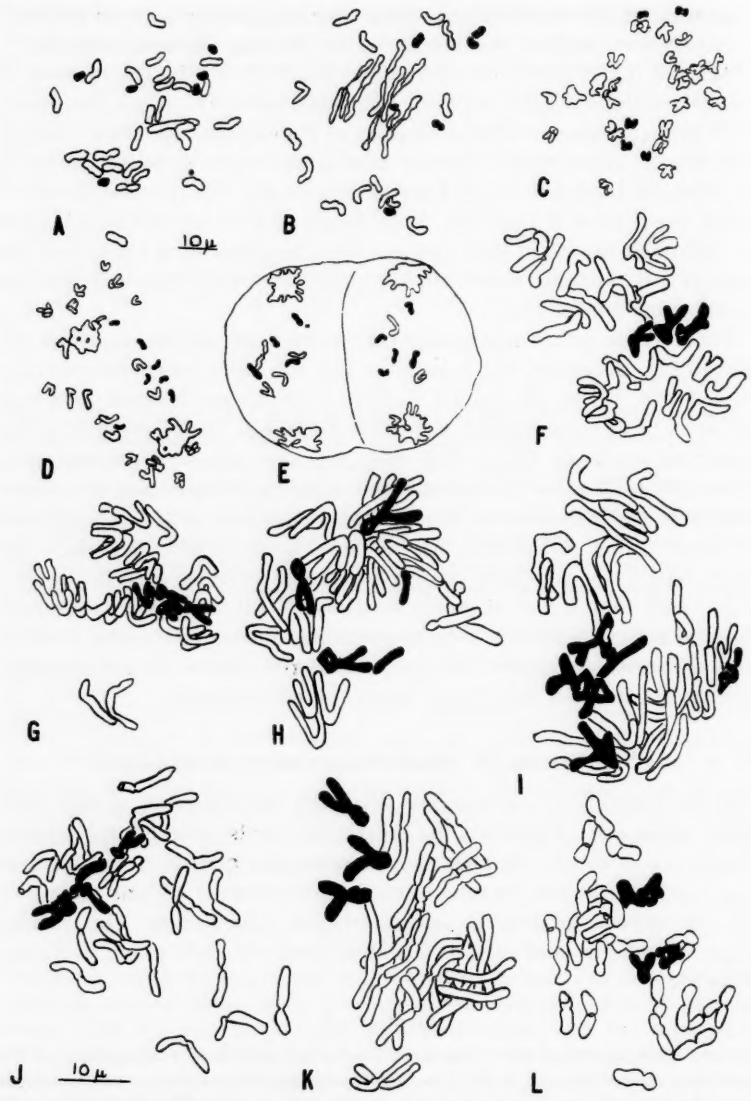

Fig. 5. Meiosis and first pollen mitosis in 4 n+6 standard accessory chromosome rye plants. — A. Metaphase I with a large amount of nonconjugation. 24 normal I+6 st. acc. chr. I+1 normal II. — B. Low amount of metaphase I conjugation. 6 acc. chr. I+15 I+5 II+1 III. — C. Anaphase I, tripolar cell with lagging chromosomes. — D.

TABLE 20. *Metaphase I associations of acc. chr. in 4n+6 st. acc. chr. plants.*

Plant no.	Types of acc. chr. associations								Total no. of cells	Cells with normal I's
	6 I	1 II + 4 I	2 II + 2 I	3 II	1 IV + 2 I	1 III + 3 I	1 III + 1 II + 1 I	1 IV + 1 II		
49—3	23.5 %	33.3 %	31.4 %	3.9 %	5.9 %	2.0 %			51	39.2 %
51—3[1]	4.1	38.8	40.8	6.1	0	6.1	4.1 %		49	34.7
49—5	3.8	13.5	26.9	30.8	5.8	3.8	3.8	11.5 %	52	9.6
41—2	76	22	2						50	100

[1] sum of two slides.

were lagging as were seen as M I univalents. A few cells possessed dots, but it is not known whether they originated from accessory chromosomes or normal chromosomes. A II cells had more accessory chromosome laggards than A I and as many as six were seen in the two plates. The per cent of normal chromosomes laggards and dots is almost the same as in A I.

The modal class of the first pollen anaphase cells was three standard accessory chromosomes. All the cells had accessory chromosomes, but none contained all six. All the accessory chromosomes in these cells showed non-disjunction. A fairly regular division of thirteen normal chromosomes is shown in Fig. 5 F although the arrangement at the upper pole was slightly irregular in contrast to the lower pole. Three standard accessory chromosomes were undergoing non-disjunction. Several types of abnormal cells were seen. In many cells the chromosomes were scattered and showed little or no anaphase movement. One such cell with five accessory chromosomes, all undergoing non-disjunction, is shown in Fig. 5 J. Some of these chromosomes appeared to have their centromeres directed towards the poles indicating that there was probably some spindle action. Many metaphase and anaphase cells were also very contracted. These cells might have the chromosomes lying parallel to each other like ski-pairs or they might be scattered (Fig. 5 L). Many of these cells

An A I cell with 3 1/2 acc. chr.+3 1/2 (?) normal chr. lagging. — E. An A II cell showing at left 4 acc. chr.+1 dot+4 normal chr. lagging; and at right 5 acc. chr.+2 normal chr. lagging. — F. Pollen anaphase with 13—13 distribution of the normal chr. and non-disjunction of 3 acc. chr. Upper pole-chromosomes slightly scattered. — G. An anaphase with 4 acc. chr. (non-disjunction) and regular distribution of 13 normal chr. but 1 normal divided chr. lies off the plate at the bottom of the cell. — H. Three acc. chr. showing non-disjunction and 1 separated. Abnormal anaphase separation with most normal chr. going to one pole. — I. Unequal anaphase division (11—17) of the normal chromosomes; 6 acc. chr. with non-disjunction. — J. Chromosomes scattered in a cell with 5 acc. chr. — non-disjunction. — K. Ski-like pairs of 13 normal chromosomes+3 acc. chr. — non-disjunction. — L. Four acc. chr. with non-disjunction+26 very contracted normal chr. No evidence of anaphase movement. — Plants 41—2 (Figs. A—E, G, I); 49—3 (F, J, L); and 51—3 (H, K). Acc. chr. in black. Figs. A—E magnified according to upper scale and Figs. F—L by lower scale.

TABLE 21. *Per cent lagging accessory chromosomes in 4n+6 st. acc. chr. plants at anaphase I.*

Plant no.	Number of lagging acc. chr.											Total no. of cells	% cells with		
	0	1/2	1	1 1/2	2	2 1/2	3	3 1/2	4	4 1/2	5		dots	lagging normal chr.	bridges
	%	%	%	%	%	%	%	%	%	%	%		%	%	%
49—3[1]	40.4	5.8	19.2	1.9	25.0	0	1.9	0	3.8	0	1.9	52	5.8	34.6	0
49—5	68	2	26	0	4							50	0	8	8
41—2															
2 polar	18	14	32	4	6	6	6	6	6	2		50	2	90	20
3 polar	0	0	100									4	25	75	25

[1] sum of two slides.

appeared to lack spindle action. The chromosomes also sometimes stained lighter and they might have a peculiar shape. In a few cells normal chromosomes were located off the plate, to one side, or else above the poles.

Plant 51—3

This plant had a higher amount of M I conjugation since 96 % of the cells had some associations. These occurred as multivalents in 10 % of the cells. Some cells had complete conjugation. About the same number of normal chromosomes compared with the preceding plant were univalents. In one cell a bivalent was located at an angle to the spindle. Some early prophase cells on the same slide were completely fragmented. A few diplotene cells were also pulled out, and more metaphase cells were clumped and impossible to analyse than in the other plants.

The next stage that was studied in this plant was pollen metaphase. The distribution of forty-two cells was 0, 4.8, 21.4, 45.2, 21.4, and 7.1 % for zero to five accessory chromosomes. The anaphase distribution was about the same except that no cells with five accessory chromosomes were seen. Abnormal cells had ski-pairs with the normal chromosomes separated but still lying parallel to each other (Fig. 5 K). This cell had thirteen normal pairs and three accessory chromosomes which showed non-disjunction. Another abnormal cell is shown in Fig. 5 H. The normal chromosomes were divided into two unequal groups of eight and eighteen chromosomes and there were three accessory chromosomes undergoing non-disjunction and one separated. There were also the "polar-view" cells which might lead to restitution nuclei. Some cells had chromosomes which lagged behind the others, were located to the side, or had reached the poles earlier.

Plant 49—5

Most of the M I cells showed some associations in this plant as in the preceding plant since only 4 % of the cells had all the accessory chromosomes as

TABLE 22. *Anaphase II. Per cent of cells with lagging chromosomes in 4n+6 st. acc. chr. plants. Sum of laggards in both plates.*

Plant no.	No. of chr. laggards											No. of cells	% cells with		
	0	1	2	3	4	5	6	7	8	9	10		dots	lagging normal chr.	bridges
	%	%	%	%	%	%	%	%	%	%	%		%	%	%
49—3	16	12	28	20	10	6	8					50	2	36	0
49—5	59.6	3.8	19.2	1.9	15.4							52	0	17.3	5.8
41—2	8	8	12	12	14	8	12	12	12	0	2	50	2	96	10

univalents. However, in this plant may cells had 3 II's or multivalents. The number of normal chromosome I's was reduced compared with the preceding plants. The high amount of conjugation at M I was reflected by fewer A I cells with lagging chromosomes. Most cells were without laggards and even those that had laggards usually had only one. Only 8 % of the normal chromosomes were lagging, which is close to the M I value for normal univalents. A few cells had bridges. One cell had an accessory chromosome and two normal chromosome bridges. At A II there were many cells without accessory laggards, but as many as four were seen in both plates. More lagging normal chromosomes were present here than at A I. Also, about 6 % of the cells had bridges.

In anaphase of the first pollen mitosis, most of the cells had three accessory chromosomes which showed non-disjunction. The remainder were divided among those cells with two accessory chromosomes and those with four. This result is expected since there was more pairing at M I and fewer laggards at A I and A II. Thus, there would be more cells with three accessory chromosomes and a narrower range of accessories. More cells would have two accessory chromosomes than four since the accessory chromosomes might lag and become lost. Only two abnormal cells were seen in this plant. Both had three accessory chromosomes undergoing non-disjunction. One cell had all its chromosomes scattered with some still appearing as ski-pairs. The other cell had an irregular distribution of the normal chromosomes into two groups of nine and seventeen.

Plant 41—2

A high per cent of M I cells had the accessory chromosomes as univalents. This value was much higher than in the other three plants. Two cells with a low amount of conjugation not only of the accessory chromosomes but also of the normal chromosomes can be seen in Figs. 5 A and B. There was only one normal chromosome II in Fig. 5 A, whereas there was one multivalent and several bivalents in Fig. 5 B. All of the cells had normal univalents. Also, bivalents might be twisted or off the plate. Accessory univalents occurred in some early diakinesis cells and the nucleolus might still be visible. All stages of division were seen on a slide, indicating some disturbances in timing. Four

TABLE 23. *Per cent of cells showing non-disjunction of accessory chromosomes at anaphase of the first pollen mitosis in 4n+6 st. acc. chr. plants.*

Plant no.	No. of slides	No. of acc. chromosomes: 0	1 non-disj.	2 non-disj.	2 both sep.	3 all non-disj.	4 all non-disj.	4 3 non-disj. 1 sep.	5 all non-disj.	6 all non-disj.	Total no. of cells
49—3	5	0	6.4 %	32.2 %	0	41.9 %	16.1 %	0	3.2 %		31
51—3	7	0	6.2	12.5	3.1 %	59.4	9.3	9.3 %			32
49—5	2	0	0	20.7	0	72.4	6.9				29
41—2	5	15.4 %	11.5	30.8	0	30.8	7.7	0	0	3.8 %	26

tripolar cells were seen at anaphase I. All these cells had one lagging accessory chromosome and some had dots, bridges, and lagging normal chromosomes. Fig. 5 C shows one such tripolar cell. Eighteen per cent of the bipolar cells had no laggards corresponding to the high number of M I cells with accessory chromosome univalents. There was also a high number of lagging normal chromosomes. Some cells showed dots and bridges. A bipolar cell (Fig. 5 D) had 3 1/2 lagging accessory chromosomes which had divided and at least that many normal chromosomes. It is hard to say in this cell exactly how many chromosomes were lagging since many normal chromosomes were scattered above each pole. One octaploid cell was also seen. Many of the A I laggards divided and regularly went to the poles since few interphase cells seemed to have lagging chromosomes. Few A II cells were without laggards, and some had as many as ten lagging accessory chromosomes. Most cells also had normal chromosome laggards and a few also had dots and bridges. Fig. 5 E shows an A II cell with a dot in addition to the lagging accessory chromosomes and normal chromosomes.

In pollen anaphase a few cells had separated accessory chromosomes. This plant had a wide range of accessory chromosomes since some cells were

TABLE 24. *Transmission of accessory chromosomes to the progeny of intercrossed plants with 4n+6 st. acc. chr. in their root tips.*

Female parent	No. acc. chr. in the progeny: 0–3	4	5	6	7	8	9	10	11	12	Total no. of plants
49—3	0	0	1	0	0	1	0	0	0	2	4
49—5	0	0	0	1[1]	0	1	0	1	0	1	4
41—2	0	1	0	1	0	0	0	1			3
%	0	9.1	9.1	18.2	0	18.2	0	18.2	0	27.3	11

[1] contains in addition 1 small iso-chr.

missing accessory chromosomes and another had all six which showed non-disjunction (Fig. 5 I). The wider range in this plant probably reflects the higher number of accessory chromosome univalents. The cell with six accessory chromosomes (Fig. 5 I) also had an abnormal unequal eleven—seventeen distribution of the normal chromosomes. Another cell (Fig. 5 G) had a regular division of thirteen normal chromosomes, whereas one divided normal chromosome lay off the plate and was located at the bottom of the cell. It is dubious whether it would be included in the lower polar group. Some cells were seen with ski-like pairs which did not always appear to contain accessory chromosomes.

Thus, tetraploid plants with six standard accessory chromosomes showed much variability. Yet, the four plants at the various stages seemed to show the same relative frequencies in respect to each other. A larger amount of pairing of the accessories and normal chromosomes at M I showed fewer cells with laggards at A I and A II. Fewer laggards seemed to be present at A II than A I, although they had about the same relative values. Pollen metaphase in plant 51—3 seemed to agree with the pollen anaphase counts. The first pollen anaphase divisions in three of the plants seemed to agree quite well, whereas the fourth plant (41—2) had a wider range than would be expected because of the greater irregularities. About 32 % of the anaphase cells showed abnormalities.

Transmission of the accessory chromosomes must be quite good since three plants out of a population of only eleven had twelve accessory chromosomes. All the plants except for one plant with five accessory chromosomes had even chromosome numbers. There was also a wide range in numbers for such a small population. The low seed set in these plants explains the small population. The higher number of abnormal cells might explain the reduced seed setting although since there were three plants with twelve accessory chromosomes, this relationship is not absolutely correlated (*i.e.* higher numbers of accessory chromosomes in the pollen giving abnormal cells which are not viable). The mechanism for increase must be quite effective since the mean number of accessory chromosomes increased from 2.70 accessory chromosomes at pollen anaphase to 8.45 accessory chromosomes in the progeny population. The small population size, however, probably unduly influences the average.

4. Plants with five and seven accessory chromosomes

Only pollen anaphase counts were made in these two groups. When they were compared (Table 25), a higher number of accessory chromo-

TABLE 25. *Anaphase of first pollen mitosis in 4n+5 standard accessory chromosomes and 4n+7 st. acc. chr. plants. Per cent of cells showing non-disjunction of acc. chromosomes.*

	No. of slides	No. of acc. chr.							Total no. of cells
		0	1	2		3		4	
			non-disj.	non-disj.	1 nondisj. 1 sep.	non-disj.	2 nondisj. 1 sep.	non-disj.	
4n + 5 acc.	4	5.4 %	10.7 %	41.0 %	7.1 %	35.7 %			56
4n + 7 acc.	3	0	0	21.6	0	52.9	2.0 %	23.5 %	51

somes were present in the pollen of plants with seven accessory chromosomes. In plants with five accessory chromosomes, zero to three accessories were seen in the pollen. Most of the accessory chromosomes showed typical non-disjunction; however, a few had separated. About seven per cent of the cells showed abnormalities. Some abnormal cells might have ski-like chromosomes, others might lead to restitution nuclei, and some had lagging, scattered or twisted chromosomes which might be off the plate.

The plant with seven accessory chromosomes had a much higher per cent of abnormalities (37 %). These were of the same type as found in the previous plant. In addition, some cells had an unequal division of the normal chromosomes which might also be contracted. Either two, three, or four accessory chromosomes were seen in the pollen. The modal class was three and only one of the cells had an accessory chromosome that had separated.

5. Plants with eight accessory chromosomes

The tetraploid plant, 40—5, which was used for a study of pollen meiosis, had eight standard accessory chromosomes plus two small iso-chromosomes. At metaphase I there were three types of accessory pairing possible: pairing among the standard accessory chromosomes, pairing among the small iso-chromosomes, or hetero-pairing between the standard accessory chromosomes and a small iso-chromosome. Since there were so many accessory chromosomes, many different types of associations could be expected and were found (Table 26). Only four per cent of the cells lacked conjugation, and nine per cent had complete conjugation of the standard accessory chromosomes. There was only a small amount of hetero-associations and of pairing among the small iso-chromosomes. Accessory multivalents were seen in many cells. Fig.

TABLE 26. *Amount of pairing of the accessory chromosomes found in 105 metaphase I pollen mother cells of plant 40—5* [1]

Amt. of pairing of acc. chr.			% of cells	Amt. of pairing of acc. chr.			% of cells
st. acc. chr.	hetero-pairing [2]	small iso-chr.		st. acc. chr.	hetero-pairing [2]	small iso-chr.	
0	0	0	3.8	3 II	0	0	20.0
0	1 II	0	1.0	2 II + 1 III	0	0	2.8
1 II	0	0	14.5	2 II + 1 III	1 II	0	1.0
1 II	0	1 II	1.0	1 II + 1 IV	0	0	11.4
1 II	1 II	0	1.0	1 II + 1 IV	1 II	0	1.0
1 III	0	0	4.8	1 II + 1 V	0	0	1.0
1 IV	0	0	1.9	1 III + 1 IV	0	0	1.0
2 II	0	0	13.3	4 II	0	0	3.8
2 II	0	1 II	1.0	2 II + 1 IV	0	0	2.8
2 II	1 II	0	1.9	2 II + 1 IV	1 II	0	1.0
1 II	1 III	0	1.0	2 IV	0	0	1.0
1 II + 1 III	0	0	8.6				

[1] This tetraploid plant had 8 standard acc. chr. + 2 small iso-chromosomes in the root tips.

[2] Association composed of at least one st. acc. chr. and one small iso-chr.

6 A shows a M I plate with the accessories associated to form a trivalent and a bivalent in addition to the univalents. There was a very low amount of association between the normal chromosomes in this cell. It was sometimes hard to tell if hetero-pairing was actually present or if it was only stickiness. Only definite hetero-associations were classified. Some cells had Y-shaped spindles (Fig. 6 B), or two or three plates. Two M I cells also had reduced chromosome numbers. Since a large number of accessory and normal chromosomes were unpaired, there were many laggards at A I (Table 27). Up to six accessory chromosomes might lag at A I although 13 % of the bipolar cells were free from accessory chromosome laggards. Fig. 6 E shows a cell with two lagging standard accessory chromosomes and one small iso-chromosome plus five lagging normal chromosomes. Many of these laggards had divided by the end of A I. One third of the cells had tripolar divisions. Both types of divisions had about the same per cent of lagging iso-chromosomes, bridges, and normal chromosome laggards. There was a similar range and distribution of accessory chromosomes in both types of divisions. Fig. 6 C shows an A I cell where the chromosomes appeared to be distributed to five poles. Fewer A II cells resulting from tripolar A I divisions were recorded than bipolar since tripolar A I cells would divide to form

A
B
C
D
10μ
E
F
G
H
10μ
J
10μ
I
K
L

several A II cells which were easily broken apart and therefore had to be discarded (Table 28). From zero to nine accessory chromosomes were lagging in the A II plates of normally dividing cells, whereas only two to five accessory chromosomes were seen in the "tripolar A I cells". A high number of iso-chromosomes lagged in both types. An abnormal cell is shown in Fig. 6 D. The small M II cell is almost separated from the larger A II cell which has five poles with the chromosomes rather irregularily distributed and with many laggards. One hexad composed of six equal sized nuclei was seen.

Fifty-nine pollen metaphase cells were scored and had from one to seven accessory chromosomes distributed as follows: 6, 13, 24, 8, 5, 2, and 1. The range at pollen anaphase was from one to six accessory chromosomes (Table 29). All the accessory chromosomes in these cells underwent non-disjunction; the modal class was four accessory chromosomes. One cell with four accessory chromosomes (Fig. 6 G) appeared normal, whereas another cell with only three accessory chromosomes was abnormal (Fig. 6 F). The latter cell had two divided normal chromosomes located over one pole while nine other chromosomes divided normally. Some cells had ski-like chromosome pairs either located close together (Figs. 6 H and I) or scattered (Fig. 6 K). Fig. 6 H appears to have the chromosomes separated into two groups although pairing was still very close. Six accessory chromosomes, all undergoing non-disjunction, were present in this cell. Fig. 6 I had two iso-chromosomes in addition to the standard accessory chromosomes. An abnormal

Fig. 6. Tetraploid rye plants with 8 standard accessory chromosomes and 2 small iso-chromosomes. Meiosis and first pollen mitosis. — A. Metaphase I plate with 1 III+I II+3 I st. acc. chr. associations+2 small iso-chr. and 11 normal chr. I's. — B. Y-shaped spindle. Acc. chr. associations: 6 I+1 II+2 sm. iso-chr. I. Normal chr: 2 I+11 II+1 IV. — C. A I cell with the chromosomes apparently divided into five groups. 8 st. acc. chr. I+2 sm. iso-chr. I. — D. Abnormal A II cell with a small cell at right almost cut off. The small cell is in M II with 6 normal chr. and 1 sm. iso-chr. The left cell has five poles at right. 1 st. acc. chr.+1 sm. iso-chr.+5 normal chr. laggards, and at left 1 st. acc. chr.+2 sm. iso-chr. laggards. — E. A I cell with 5 lagging normal chr.+2 st. acc. chr.+1 sm. iso-chr. — F. Pollen anaphase with regular division of 9 normal chr. with 2 normal divided chr. at top of cell. 3 st. acc. chr. showing non-disjunction. — G. Normal anaphase division of 15 normal chromosomes with 4 st. acc. chr. present and undergoing non-disjunction. — H. Six acc. chr. with non-disjunction and 15 ski-like pairs of normal chr. — I. Another ski-like division of 13 normal chr.: 4 st. acc. chr.+2 sm. iso-chr. (non-disjunction). — J. An abnormal anaphase cell. Normal chr. appear corroded. 4 st. acc. chr. stretched and pulled out. — K. Scattered 28 normal chr.+5 acc. chr. (non-disjunction). — L. Telophase of pollen mitosis showing scattered chromosomes. — Plants 40—5 (Figs. A—F, I—L) and 49—13 (G, H). Acc. chr. shown in black. Figs. A—E magnified according to upper scale, F—K by lower left, and L by lower right scale.

TABLE 27. *Anaphase I. Per cent PMC's with lagging accessory chromosomes in 4n+8 standard acc. chr.+2 small iso-chr. plants.*

Plant no.	Number of lagging acc. chr.													No. of cells	% of cells with		
	0	1/2	1	1 1/2	2	2 1/2	3	3 1/2	4	4 1/2	5	5 1/2	6		lagging iso-chr. [2]	normal chr. lag.	bridges
	%	%	%	%	%	%	%	%	%	%	%	%	%		%	%	%
40—5 [1]																	
2 polar	12.7	4.2	22.5	2.8	28.1	2.8	14.1	1.4	8.4	0	1.4	0	1.4	71	71.8	85.9	2.8
3 polar	14.7	8.8	23.5	11.8	14.7	2.9	17.6	0	2.9	0	2.9			34	70.6	85.3	5.9

[1] sum of three slides.

[2] from 1/2 to 2 small iso-chr. lagging.

TABLE 28. *Anaphase II. Per cent of cells with lagging chromosomes in 4n+8 standard acc. chr.+2 small iso-chr. plants. Sum of laggards in all plates.*

Plant no.	Number of lagging acc. chr.										No. of cells	% of cells with	
	0	1	2	3	4	5	6	7	8	9		lagging sm. iso-chr.[2]	lagging normal chr.
	%	%	%	%	%	%	%	%	%	%		%	%
40—5[1]													
2 polar	16.7	8.3	8.3	22.2	16.7	8.3	8.3	0	5.6	5.6	36	77.8	86.1
3 polar AI cells	0	0	16.7	16.7	16.7	50.0					6	66.7	100

[1] sum of two slides.
[2] from 1/2 to 2 small iso-chr. lagging.

anaphase cell is shown in Fig. 6 J. The normal chromosomes appeared corroded and the accessory chromosomes were stretched and pulled out. The lower accessory chromosome had the short arms pulled out while the long arms remained together and parallel. They were held together by the special segment which was also stretched. A few cells also had contracted chromosomes, and some cells looked like restitution nuclei. Other cells had scattered chromosomes and appeared to have no organized spindles. Several telophase cells (Fig. 6 L) which probably resulted from scattering of the anaphase chromosomes were seen. Bridges were also seen in some of these cells. About 84 % of the anaphase cells showed abnormalities. Although several plants were in the intercrossing group,

TABLE 29. *Per cent of cells showing non-disjunction of standard accessory chromosomes in anaphase of first pollen mitosis in 4n+8 st. acc. chr.+2 small iso-chr. plants.*

Plant no.	No. of slides	Number of acc. chr.							Total no. of cells
		0	1	2	3	4	5	6	
				non-disj.	non-disj-	non-disj.	non-disj.	non-disj.	
49—13	5	0	0	7.4 %	29.6 %	37.0 %	14.8 %	11.1 %	27
40—5—B	4	0	0	3.8[1]	19.2[1]	42.3[1]	26.9[1]	7.7	26
40—5—C	6	0	0	8.3[1]	12.5[1]	54.2[1]	20.8	4.2	24

[1] plus 1—2 other pieces.

only one seed was obtained. This plant had six standard accessory chromosomes.

6. Plants with twelve standard accessory chromosomes

Only twelve pollen metaphase divisions were obtained from this plant. They showed from four to ten accessory chromosomes distributed as follows: 2, 1, 2, 2, 4, 0, and 1. The cell with ten accessories appeared to have pulled out and sticky chromosomes. Several nucleoli were present in another cell. There were many empty grains and many irregular shaped grains on the slides. Some of these grains were large enough to have come from restitution nuclei.

7. Diploid rye at first pollen anaphase

A population of diploid rye from Transbaikal was examined at first pollen anaphase to obtain an estimate of the frequency of the accessory chromosomes. This was desirable as the tetraploid material had been derived from this variety. Out of 127 plants, 101 had no accessory chromosomes (Table 30). The others had pollen grains with one or two accessory chromosomes. Some were of the standard type, some a small type, or others dot-like in appearance. The population mean was 0.22 accessory chromosomes per plant.

The pollen grains in the different plants were studied for the frequency of accessory chromosomes and for abnormalities. When a plant was found to contain one accessory chromosome, further slides were made and studied to see if two might be present. When a plant had only one standard accessory chromosome 91 % of the pollen grains contained one accessory chromosome (Table 31). Less than one per cent of the cells had separated accessory chromosomes. The few cells from the plant with the small accessory chromosome had about twice as many without an accessory chromosome as in the preceding class. Many more cells also had the accessory chromosome separated. Three plants had

TABLE 30. *Number of acc. chr. in a diploid population of Transbaikal rye determined at anaphase of first pollen division.*

	No. of acc. chr.							Total no. of plants
	0	0 or 1	1 dot	1	1 sm. acc.	1 st. acc. + 1 sm. acc.	2 acc.?	
No. of plants	101	2	1?	18	1	1	3	127

TABLE 31. *Number of diploid rye cells at anaphase of first pollen division which show non-disjunction of acc. chr.*

Type of plants	No. of plants	No. of acc. chr.								Total no. of cells
		0	1 or 2 small	1		1 st. + 2 small	1 st. + 1 small	2		
				non-disj.	sepa-rated			both non-disj.	one sepa-rated	
Only one st. acc. chr. observed	15	8.6 %		90.8 %	0.6 %					175
1 sm. acc.	1	16.6		66.7	16.6					6
2 acc. chr. observed in at least 1 pollen gr.	3	1.9	0.6 %	91.5	0.6	1.2 %	2.5 %	1.2 %	0.3 %	316
1 st. acc. + 1 sm. acc.	1	1.4	0	82.5	0.5	2.8	0.9	9.0	2.8	211

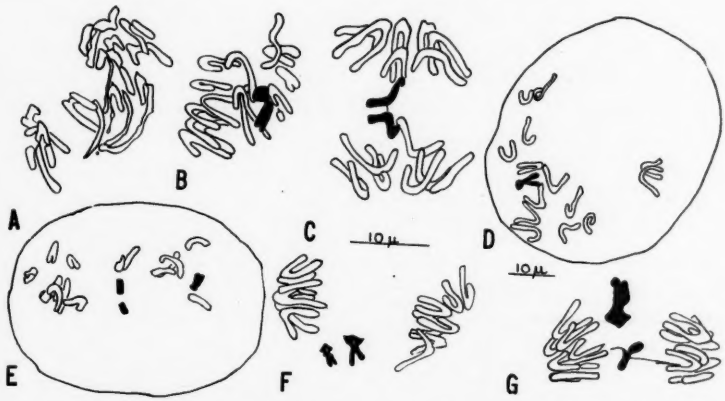

Fig. 7. First pollen mitosis in diploid rye. — A. Abnormal twisted anaphase. Cytoplasm was stained deeper than usual. No acc. chr. were present in this plant. — B. Twisted division, 1 st. acc. chr. (non-disjunction). — C. Regular division of 7 normal chr. Acc. chr. in process of separation. — D. Abnormal distribution of the normal chr. One acc. chr. undergoing non-disjunction. — E. Odd division of normal chr. which were very contracted. Probably 3 acc. chr. — F. Normal division with 1 st. acc. chr. and one smaller acc. chr. — both showing non-disjunction. — G. Same plant as F. One acc. chr. showing non-disjunction and one small acc. chr. separating. Acc. chr. shown in black. — Figs. A—C, F, and G magnified by left scale and D, E by right scale.

two accessory chromosomes in at least one pollen grain. These plants contained many kinds of accessory chromosomes. Fig. 7 C shows one accessory chromosome in the process of separation. When a standard accessory chromosome and a small accessory chromosome were both present in the same plant, many cells contained both chromosomes. Most of these showed non-disjunction (Fig. 7 F) but Fig. 7 G shows a small accessory chromosome in process of separation.

Some abnormalities were seen in the pollen grains of the diploids. These were present in four per cent of the cells without accessory chromosomes. Fig. 7 A shows a twisted division with stretched chromosomes. The cytoplasm was also stained deeper than usual. Another twisted division with one accessory chromosome is shown in Fig. 7 B. One cell also had a piece of stretched-out chromatin remaining in the cytoplasm between the two poles. One case of an unequal six—eight division of the normal chromosomes occurred. The normal chromosomes might be scattered, pulled out, fragmented, or sticky. Fig. 7 D shows a cell with scattered normal chromosomes and one accessory chromosome undergoing non-disjunction. One anaphase was very sticky and had jelly-like chromosomes which could not be counted. Another abnormal cell was very contracted and, therefore, hard to count accurately (Fig. 7 E). There were probably three accessory chromosomes in this cell, and the normal chromosomes were scattered. One anaphase cell had its chromosomes located in three groups with a nucleolus in one. Since the chromosomes were clumped, the exact numbers could not be counted and no accessory chromosomes could be recognized. The number of abnormal pollen grains in plants with only one accessory chromosome did not differ significantly from those plants which had more than one accessory chromosome in a few pollen grains.

IV. DISCUSSION

The appearance of the accessories at the various meiotic and mitotic stages has been examined for the tetraploid groups with varying numbers of accessory chromosomes. Considerable variability was noted between the different plants; however, general patterns were seen. These tetraploid groups can be compared with the diploids in respect to the frequencies of the accessory chromosomes and the rates of transmission. The frequencies and types of abnormal divisions in the first pollen anaphase can be similarly studied and compared.

1. Comparison between tetraploid and diploid frequencies

The number of accessory chromosomes in the tetraploid C_8 and the diploid populations of Transbaikal rye differed (Tables 1 and 30). The tetraploids appeared to tolerate more accessory chromosomes in a plant then the diploids. The most accessory chromosomes seen per plant in any of the diploid populations were six, whereas the tetraploid possessed up to twelve. Intercrosses of diploid Wasa II rye plants with six accessories showed that it was possible to obtain as many as ten accessory chromosomes (MÜNTZING, 1954). Nine diploid populations including the strain from Transbaikal accumulated accessory chromosomes at even numbers which showed that non-disjunction must be quite effective (ibid.). The tetraploid Transbaikal population did not have a corresponding concentration at the even values and did not show the marked increase in average number of accessory chromosomes characteristic of most diploid strains. Another interesting difference is that there appeared to be more deviating types of accessory chromosomes in the tetraploid population than in the diploids.

Since there were wide variations between the different tetraploid groups, only general comparisons can be made between the stages in these groups and in the diploids. Tetraploid M I cells had increased pairing of accessories as more were present. More multivalents were seen although the highest observed was a quadrivalent. The addition of an iso-chromosome in the 4 n+2 standard accessory chromosome group reduced M I pairing of the standard accessory chromosomes. The effect of the iso-chromosome could be specific to this chromosome, the effect of an additional chromosome, or of an odd number of chromosomes in the cell. Yet, in the 4 n+4 standard accessory chromosome group, pairing did not appear to be reduced in plant 40—3, which was missing one accessory chromosome, from plant 41—3 with four accessories. Pairing was, however, considerably higher in the other two plants. The small iso-chromosome apparently does not interfere mechanically with pairing since it paired only to a limited extent with the standard accessory chromosomes in the tetraploids (Tables 3, 26) and was usually eliminated (Table 9). In the 4 n+8 standard accessory chromosome plant, there did not appear to be a decrease in pairing of the standard accessory chromosomes in spite of the presence of the two small iso-chromosomes. However, the presence of so many standard accessories in relation to the iso-chromosomes might have influenced the effect of the iso-chromosome. Since all the collections were made at about the same time, it is not likely to be an environmental effect.

BOSEMARK (1956) found in *Festuca* a positive influence of a small accessory chromosome on the non-disjunction mechanism of the standard type at pollen mitosis. He explained this difference on the basis of a compensation for the shortage or a lack of gene products necessary for full manifestation of non-disjunction. It does not appear that the effect of the iso-chromosome is caused by a M I competition for genic substances since plants with eight standard accessory chromosomes seem to pair as well in the diploids (HÅKANSSON, 1957) as in the tetraploid with the two small additional iso-chromosomes.

Metaphase pairing of accessory chromosomes in the diploids with two accessory chromosomes depends on the variety of rye. A typical variety, Wasa II had a high amount of pairing with only 11.1 % of the accessory chromosomes unpaired. Eight Östgöta Gråråg plants averaged 61.8 % unpaired accessory chromosomes with a range from 45 to 86 %. However, this latter variety is not typical of diploid rye with accessory chromosomes. HÅKANSSON (1957) states that in diploid plants of this variety with two and four standard accessory chromosomes, bivalents were formed at early diplotene and had separated by diakinesis. MÜNTZING and LIMA-DE-FARIA (1949) also showed that there were no differences in pachytene pairing of the two varieties and the poor metaphase pairing in Östgöta Gråråg must be caused by post-pachytene conditions viz., a reduced chiasma frequency. The tetraploid plants with two accessory chromosomes behaved like Östgöta Gråråg, especially the earlier study of MÜNTZING and PRAKKEN (1941). Diplotene stages of the tetraploids were not examined to see if there was a similar separation which could account for the poor meiotic pairing in the tetraploids.

Variability between plants could be attributed to two causes: one is environmental and the second might be the physiology of the host plant. MÜNTZING (1951 b) found that both factors caused variations in pairing of rye iso-fragments. There were even differences between locules of the same spikelet. In the one diploid plant with six accessories, HÅKANSSON (1957) found variability in the amount of pairing, even in anthers from the same plant. The tetraploids also showed wide variations in pairing from plant to plant (Table 20). The variation between the two varieties of rye has already been discussed. BOSEMARK (1954 a) also found similar differences between plants of *Festuca pratensis*. He concluded that the physiology of the host plant influenced the behavior of the accessories more than the environment. He also considered inherent differences between the accessories as a third factor which might lead to these differences in behavior.

The number of A I lagging accessory chromosomes in the tetraploid group with two accessory chromosomes was quite variable and was related to metaphase pairing. If an average is taken from these plants, only about 25 % of the cells did not have laggards.

In a diploid population of Östgöta Gråråg, about 47 % of the cells were without laggards (MÜNTZING and PRAKKEN, 1941). Also, many of the laggards were divided in the tetraploids, whereas in the diploids they were randomly distributed (ibid.). The small iso-chromosome usually lagged and divided at A I in both 2n's (MÜNTZING and LIMA-DE-FARIA, 1953) and 4n's. The frequency of lagging iso-chromosomes was about the same in plants with two standard accessory chromosomes as in those with eight accessory chromosomes. However, there was only one in the first case and two in the latter. Only one tetraploid plant in the group with four accessory chromosomes showed fewer cells without laggards than plants with two accessory chromosomes. The two plants, 40—3 and 40—10, which had tripolar spindles had many cells without accessory chromosome laggards. Two of the three tetraploid plants with six accessory chromosomes also had many cells without accessory chromosome laggards. This percentage, however, dropped in 4n plants with eight accessory chromosomes.

The number of A I bridges was low in all groups. A bridge was also seen in the diploids (HÅKANSSON, 1957). The frequencies of lagging normal chromosomes in the tetraploids varied from plant to plant. However, the values seemed to be higher in plants with tripolar cells and the plant with eight standard accessory chromosomes. Interphase in the tetraploid groups with two and four accessory chromosomes showed that most cells were without micronuclei. Therefore, many A I laggards must have been included in the telophase nuclei. BAUER (1931) also found in *Tipula paludosa* a delayed movement of the supernumerary chromosomes which sometimes were distributed the length of the spindle. In the second division they might be more or less uniformly distributed to the cells or the division might be surpressed.

In anaphase II diploid cells, HÅKANSSON (1957) reported that division was regular, but the chromatids from first anaphase lagged. Thus, it might be expected that the results from A I would be correlated with A II. The results in all four tetraploid groups showed that when the number of cells without laggards are compared; this is the case. Usually, the two values for a plant were quite close or else the A II value would be lower. Also, about the same per cent of lagging iso-chromosomes were seen at A II as A I. Tetrads without micronuclei had about the

same percentage as A I and A II cells without laggards. Similar relationships occur in *Festuca pratensis* between M I, A I, A II, and tetrads (BOSEMARK, 1954 a). Normal rye chromosome laggards also showed approximately the same relationships. A few tetraploid cells had bridges like in the diploids (HÅKANSSON, 1957). He said that elimination of chromosomes in his diploids with six and eight accessory chromosomes was much less frequent than would be expected from the number of laggards. However, in the tetraploids only a few cells were without micronuclei. This value would be expected to be higher if elimination did not occur. Yet, in these cells not only would accessory chromosomes contribute to the number of micronuclei but also would normal chromosomes and iso-chromosomes. It, therefore, appears that many laggards are lost in the tetraploids and many accessory chromosomes are eliminated. Thus, in meiosis an increase in accessory chromosomes from two to eight appears to have affected the cells to a greater extent than did the change from a diploid to a tetraploid.

Metaphase and anaphase pollen divisions with varying numbers of accessory chromosomes can be compared in diploids and tetraploids. Metaphase frequencies for diploid Östgöta Gråråg plants with two standard accessories were 36, 57.6, 4.7, and 1.8 % for cells with zero, one, two and with an iso-chromosome, respectively (MÜNTZING, 1946). The corresponding values for the tetraploids of the Transbaikal strain were 48.8, 44.5, 5.1 and 1.6 %. The mean frequency of the diploids was 0.70 accessories per plant and for the tetraploids, 0.57. Thus, there does not appear to be a large difference between these two populations. However, as mentioned earlier this diploid variety is not typical of the other diploids with accessory chromosomes. Unfortunately no material of diploid Transbaikal was available for comparison with tetraploid Transbaikal, but other data (*cf.* MÜNTZING, 1954) demonstrate that meiotic elimination of accessories must be low in the diploid strain in contrast to the marked elimination occurring in the corresponding tetraploid.

MÜNTZING (1946) showed that a small iso-chromosome was present in only ten per cent of the pollen grains of a diploid plant. The one tetraploid plant with an iso-chromosome, 45—2, had it in only three per cent of the pollen. The two pollen metaphase distributions for plants with four standard accessories agreed quite closely. The mean number of accessories was 1.72 in the diploids (ibid.) and 1.69 in the tetraploids. The values in diploid Östgöta Gråråg for zero to four accessories and with an iso-chromosome were 2.1, 40.4, 44.7, 6.4, 2.1, and 4.2 %, respectively (ibid.) which agrees with the tetraploids in Table 17. The one

tetraploid plant with six accessory chromosomes in which the pollen metaphase was studied had a higher mean of 3.05 accessories. The values for the plants with eight and twelve accessory chromosomes were 3.05 and 6.75, respectively. These values are about what might be expected if there had been relatively good pairing at M I and regular movement of the chromosomes at A I and A II with inclusion of the chromosomes in the tetrad nuclei. Since these stages were not always regular, the mean values were somewhat reduced.

First pollen anaphases in the tetraploids showed that most accessory chromosomes undergo non-disjunction. Three diploid plants of Östgöta Gråråg with two standard accessory chromosomes showed 51 out of 113 cells had regular divisions, whereas the others had lagging accessories (MÜNTZING, 1946). This distribution did not differ significantly from the diploid metaphase observations. The two stages for the tetraploid plants with two accessory chromosomes showed similar agreement. If this diploid variety is compared with Wasa II the latter showed a much lower degree of meiotic elimination which influenced the number of accessory chromosomes present at the pollen divisions. The mechanism of non-disjunction in the two varieties is probably the same (MÜNTZING, 1954).

It is interesting to note the higher frequency of separated accessory chromosomes in the tetraploid plant with the small iso-chromosome. One explanation by BOSEMARK (1956) was previously described. However, these pollen grains did not have the mall iso-chromosome present, thus it appears that either the whole physiology of the plant was influenced or else an iso-chromosome in one pollen grain might affect another without it and thereby disturb the non-disjunction mechanism. Diploid plants of the same variety with four accessories were found to be a little more irregular (MÜNTZING, 1946). However, seven out of seventeen anaphases were regular while an equal number contained lagging accessories. The anaphase results of tetraploids with four standard accessory chromosomes also agree closely with the metaphase counts. The 4n's and 2n's, however, are not as close, possibly because separated accessory chromosomes may go to the poles and these cells may then be included in the counts as regular cells. Yet, this source still cannot account for all the differences although the diploid sample was small. Tetraploids with six accessories have even fewer anaphase cells minus accessories than expected. The distribution agrees quite well with the metaphase frequencies. In these plants non-disjunction was much more regular than in preceding groups. Also, in plants with five, seven,

and eight accessories non-disjunction was very regular. As the number of accessories increased, the larger the number of accessory chromosomes in the pollen — the higher the mean. These values for the 2, 4, 5, 6, 7, and 8 accessory chromosome groups were 0.53, 1.42, 2.14, 2.70, 3.02, and 4.02, respectively. These means agreed quite well with the metaphase results except the group with eight accessory chromosomes was slightly higher at anaphase than metaphase.

It is now interesting to see how the accessories were transmitted to the progenies of both tetraploids and diploids. The data for the diploid progenies of Östgöta Gråråg and the methods of calculations were taken from MÜNTZING (1945, 1946). The mean for the diploid progenies with two accessory chromosomes was about the same as the mean of the parental types. Yet as MÜNTZING, 1954, states, the tendency for numerical increase in Östgöta Gråråg is weaker than in the variety Wasa II and is primarily caused by differences in pairing and meiotic elimination of the accessories and not by differences in the non-disjunction mechanism. Diploid Transbaikal rye had an increase of accessories from 2 to 3.11 in intercrossed plants. However, the tetraploid group with two accessory chromosomes had only a mean of 0.81, whereas the original population mean was higher (1.16 accessory chromosomes). Thus it appears that *the tetraploid population has lost the ability of numerical increase*. Yet, in some plants there was an increase of accessory chromosomes.

When the offspring of diploid Östgöta Gråråg plants with two accessory chromosomes were examined, the frequency of accessory chromosomes was lower than expected from observation of pollen metaphases. There was a marked deficit of plants with four or more accessories and a surplus of plants with zero to three accessory chromosomes. A similar pattern was found in tetraploid Transbaikal. The metaphase counts were then used to calculate the expected frequency of accessories in the progeny. When a χ^2 test was made of the classes: 0, 1—2, and 3 or more accessory chromosomes, the results gave a highly significant difference between the observed and expected values. It was assumed that the behavior of the accessories was similar on the male and female sides and all the accessory chromosomes undergo non-disjunction, remain together, and are included in the generative nucleus. Since the pollen anaphase cells showed that some of the accessory chromosomes had already separated, the χ^2 test was made again, assuming that all the accessory chromosomes which still showed non-disjunction would have both chromosomes included in the generative cell and the others would

go to the poles. The result of this test was highly significant as well as when it was redone, assuming that all the chromosomes would eventually separate and go to the poles. The diploid progenies were divided into two classes: with and without accessory chromosomes, and were compared with the tetraploids using DAVIES' method (1954). There was a highly significant difference between the two groups. The transmission of the small iso-chromosome could not be compared since the number of plants in the tetraploid progeny was too small.

If the 2n and 4n groups with four accessory chromosomes are examined, the two metaphase means were similar for the diploids and the tetraploids: 1.72 and 1.69, respectively. The 4n pollen anaphase mean was only slightly lower (1.42). The tetraploid metaphase data from Table 17 were used to calculate the progeny expected on the basis of non-disjunction of all the accessory chromosomes and their inclusion in the generative nucleus. The classes 0—2, 3, 4, and 5 or over were used to compare the expected results with the actual counts obtained in the progeny. Highly significant results were obtained. The tetraploid anaphase data were then used in the calculations assuming that the chromosomes which had already separated would go to the poles and those showing non-disjunction would be included in the generative nucleus. A highly significant result was obtained from this χ^2 test as well as when the assumption was made that all the accessory chromosomes would disjoin and move normally to the poles. As in the diploids, there was a surplus of plants without or with only a few accessory chromosomes and a corresponding decrease of plants with high numbers. Intercrossed plants had progeny means of 3.43 for the diploids and 2.80 for the tetraploids. If the classes of three or fewer accessory chromosomes and three and over are compared in the 2n's and 4n's there was a significant difference (P between 0.05 and 0.01) between the two groups.

Plants with six accessory chromosomes set very few seeds in both diploids and tetraploids. Seeds from the diploids could be obtained only after open pollination and only eleven plants from the tetraploid intercrosses were obtained. The few plants in the tetraploids had a very high mean of 8.45 accessories, showing that there might be an increase of accessory chromosomes in the progeny. If the tetraploid anaphase data are used to calculate the number of accessory chromosomes expected in the progeny, assuming that there was complete non-disjunction, the expected results agreed with the counts obtained in the progeny.

It appears, therefore, that the metaphase and anaphase data cannot

be used to predict the frequency of accessory chromosomes in the progenies of either the diploids or tetraploids. Also, diploids and tetraploids even though they had similar distributions at metaphase and anaphase of the pollen divisions, were found to transmit their accessory chromosomes in a different fashion. When comparing diploids with tetraploids, the greater ability of the 4n's to tolerate more accessory chromosomes should also be considered. If the assumptions are examined, some explanation might be found to account for the discrepancies. Transmission of the accessory chromosomes on the male and female sides should probably be essentially the same in the 4n's as was shown for the 2n's. Since not all the accessory chromosomes exhibited complete non-disjunction and some separated, this factor could explain some deviation. Yet, if allowance was made for this separation by using the anaphase data, significant differences were still seen in the tetraploids. Another source of error could be some lagging of the accessory chromosomes which show non-disjunction so that they would not be included in the generative nucleus. Second pollen grain divisions would have to be studied to clarify this point.

Pollen grains in diploid plants which had accessory chromosomes were found to be retarded in their development in contrast to those without accessory chromosomes (MÜNTZING, 1949). Since the pollen grains with accessory chromosomes in diploids were retarded at earlier meiotic stages, they might also be retarded at anthesis or in fertilizing the egg cell. A similar situation was not observed in the tetraploids so it does not appear that the pollen grains carrying accessory chromosomes would be selectively eliminated because of these factors. However, this difference in development might explain some of the differences in the transmission of accessories between the diploids and tetraploids.

An environmental effect was observed by BOSEMARK (1954 b) in *Festuca pratensis*. Variations in respect to crosses, years, and even plants were found and were considered to be due to either external environment, inherent differences between different accessory chromosomes, or differences in the internal plant environment. The third alternative appeared to be the cause. To allow for a similar difference in the tetraploid rye plants, the same plants were studied in both meiosis and mitosis, and for their transmission of accessory chromosomes to the progenies. Unfortunately, not enough seeds were obtained from an individual plant to make comparisons, therefore, the data had to be summed. Since the same plants were usually involved, the data should

not have been unduly biased. Some differences were seen between plants in the separation of accessory chromosomes at anaphase (Tables 10, 18, and 23). MÜNTZING (1946) felt that the deficit of accessory chromosomes in the diploids might be caused to some extent by irregularities of the pollen mitosis but the most important cause was probably a reduced viability of gametes with many accessory chromosomes. As the number of accessory chromosomes increased, the number of abnormalities also increased (Table 32, to be discussed later). However, the tetraploids with six accessory chromosomes showed that high numbers of accessory chromosomes could be obtained and even in a small group of only eleven plants, seven had higher numbers than the parents. Thus, the pollen abnormalities in relation to accessory chromosomes should be examined as a cause of reduced viability.

2. Abnormalities in meiosis and pollen mitosis

The various abnormalities at tetraploid meiosis and pollen mitosis were compared with the types seen in diploids and with those that were previously reported for diploid rye (MÜNTZING and PRAKKEN, 1941; MÜNTZING and AKDIK, 1948 a; and HÅKANSSON, 1948 and 1957). Thirteen different categories of meiotic abnormalities ranging from minor to major disturbances were seen. These types were described and illustrated earlier. The diploid plants used in the comparisons were composed of three types: normal plants, inbred plants, and plants with accessories. About the same kinds of abnormalities appeared in both the tetraploids and diploids. Only three types were seen in the tetraploids which were not reported for the diploids: Y-shaped metaphase I plates, tripolar anaphases, and misdivision of the normal chromosomes. All three are probably interrelated. Some types in the diploids were seen only in inbred plants and in the plants with accessories; however, they were types which would be expected in diploids. These included cells with stickiness as in Fig. 4 C, bridges, lagging chromosomes giving rise to micronuclei, abnormal orientation of the bivalents, restitution nuclei, and contracted M I chromosomes. The different types in diploids were perhaps increased by the presence of accessory chromosomes or through inbreeding.

The first pollen grain divisions showed eighteen types of abnormal cells. Only seven of these were seen or described in plants without accessory chromosomes. Yet, the tetraploids had only two classes different from the diploids when plants with accessories and inbreds

TABLE 32. *Anaphase of first pollen division in rye showing the per cent of abnormalities associated with the number of accessory chromosomes.*[1]

	Number of acc. chr. in pollen							
	0	1	1 + 1 sm.	2	3	4	5	6
	%	%	%	%	%	%	%	%
2n + 1 or more acc. chr. in pollen grain	4.0 (25)[3]	9.1 (631)[3]	4.5 (22)[3]	3.3 (30)[3]				
4n + 2 acc.	3.0 (99)	7.7 (91)	—	33.3 (6)				
4n + 4 acc.	3.8 (26)	3.4 (61)	—	12.1 (58)	47.0 (17)[3]			
4n + 5 acc.[2]	0.0 (3)	0.0 (6)	—	3.7 (27)	15.0 (20)			
4n + 6 acc.	0.0 (4)	0.0 (7)	—	3.4 (29)	39.3 (61)	73.3 (15)[3]	100 (1)[3]	100 (1)[3]
4n + 7 acc.	0 (0)	0 (0)	—	0.0 (11)	25.0 (28)	100 (12)		
4n + 8 acc.	0 (0)	0 (0)	—	20.0 (5)	56.2 (16)	97.0 (34)	100 (16)	100 (6)

[1] includes both minor and major abnormalities.
[2] also had 8 cells with polar views.
[3] number of cells investigated.

were included: corrosion of the normal chromosomes and haploid cells. However, some types were seen only in plants with accessories: abnormal arrangements of the chromosomes on or off the metaphase plates, fragmentation of the cells, unequal distribution of the chromosomes, possible unipolar cells, extreme contraction of the metaphase chromosomes, tripolar divisions, and cells with no poleward movement with the chromosomes in ski-like pairs. Therefore, it appears that the type of abnormality depends more on the presence or absence of accessories than whether a plant is diploid or tetraploid.

The anaphases of the first pollen divisions were examined to determine the relation of the number of accessory chromosomes in a cell to the per cent of abnormalities and then to see what influence tetraploidy might have in relation to the diploid types. Table 32 lists the diploids and the various groups of tetraploids that were examined. The pollen grains were divided into groups depending on the number of accessory chromosomes. The per cent of abnormal cells was then calculated for each group. As the number of accessories increased, the frequency of abnormalities also increased. In the tetraploid groups with two and four accessory chromosomes in the roots, there was an increase of abnormalities when only two accessories were present in the pollen. In the groups with four and five accessories in the roots, three accessories showed four times as many abnormalities as cells with only two. In the remaining three groups, there was a very considerable increase of abnormalities in cells with three accessories. With four or more, most of the pollen grains were abnormal. However, in the cells with four accessories a few cells were normal. This result is expected because some progeny plants had as many as twelve accessory chromosomes. Therefore, either not all the cells with large numbers of accessories are abnormal or else the abnormal cells recover sufficiently to fertilize the egg cells. If more cells with many accessories could have been obtained, this question might have been answered. Also, these results might rule out the influence of abnormalities in one PMC affecting the degree of abnormality in another. There appeared to be no relation between an aneuploid number of normal chromosomes in the tetraploids and the action of the accessories. There also appeared to be no relation between the per cent of abnormalities and the presence of even and odd numbers of accessories in the pollen division although the data are meager for cells with five accessories. Even and odd numbers in the root tips of the parental plant did not seem to influence the percentages, although the odd-numbered plants had fewer cells. A limited number of diploid pollen

grains with accessories was investigated. These showed that only a very low per cent of cells with accessories were abnormal and did not appear to differ appreciably from the tetraploids. If the influence of the accessories is related to a balance of accessories to normal chromosomes, then the following comparisons should also be made: 2 n+1 acc. chr. with 4 n+2 acc. chr., and 2 n+2 acc. chr. with 4 n+4 acc. chr. The data are meager but the diploid classes zero and one accessory chromosome do not appear to differ from the 4 n+2 acc. classes. The diploid class with two accessories was slightly lower than the tetraploid with four accessories. Unfortunately, in the diploid plants, no pollen grains with more than two accessories were seen. However, HÅKANSSON (1948, 1957) had investigated diploid plants with many accessories. He found that if three or more accessories were present, the pollen division was completely upset. Thus, the number of accessories in the pollen seems to be the determining factor and is not changed by doubling the normal chromosome number.

A decrease in rye pollen fertility was observed by POPOFF (1939) in both field and greenhouse plants. This reduction was associated with an increasing number of accessories. MÜNTZING (1954) saw a 52 % reduction in the kernel yield per plot in two Transbaikal rye populations. The low-yielding group averaged 3.11 accessories, whereas the high yielding category had only 0.61 accessories. Many other plants also showed a similar decrease in fertility with an increasing number of accessories. A decrease with three or four accessories was seen in *Crepis syriaca* (CAMERON, 1934); *Sorghum purpureo-sericeum* (JANAKI-AMMAL, 1940); and *Festuca pratensis* (BOSEMARK, 1957 a). Also, in *Anthoxanthum*, ÖSTERGREN (1947) found a decreased fertility when accessories were present and RANDOLPH (1941) saw a decrease in maize only after ten to fifteen accessories. Up to three accessories proved to be favorable in *Trillium grandiflorum* (RUTISHAUSER, 1956 a). One case of male sterility caused by a single accessory chromosome was reported by PALIWAL and HYDE (1958). Paralleling these effects on fertility, other phenotypic effects were cited by many of these authors.

The action of the accessory chromosomes seems to be on the mitotic spindle and does not change with an increase of the normal chromosomes. The above summary shows that pollen grain divisions which have accessory chromosomes may not show normal spindle action. In cells with only one or two accessory chromosomes, the abnormalities may be merely a slight disarrangement of a single chromosome. However, with about three, major upsets occur. The spindle activity appears to

be stopped, resulting in the typical ski-like pairs (Fig. 5 K). The spindle action is not completely inhibited because some cells are able to recover. However, the scattering may result in unequal distribution of the chromosomes. The extreme contraction of some pollen metaphase and anaphase chromosomes may be related to the failure of the spindle to form at the proper time as SWANSON (1942) showed. In late prophase, BAJER (1958) describes a contraction stage which is associated with spindle formation. During this stage he says the spindle "gelifies" and the orientation of the spindle is determined. Perhaps the presence of the accessory chromosomes would upset one of these factors. The centromere of the accessory chromosome appears to function normally (Figs. 2 G and 6 J) and does not appear to be related to the abnormalities. If there are abnormal spindle fiber attachments, it may be interesting to use stains specific for the spindle and compare cells with high and low chromosome numbers. HÅKANSSON (1948, 1957), working with diploid rye, observed a similar effect of the accessory chromosomes on the spindle. He suggested that with two accessory chromosomes the mitotic system might be under stress and with three it breaks down. He felt that the influence of the accessories on the spindle might be less if fourteen normal chromosomes were present instead of seven since he believed that the spindle defects were caused by too many accessories in relation to the ordinary chromosomes. Yet, as was previously discussed, this does not appear to be true for the rye tetraploids.

It is possible that the accessory chromosomes affect not only the first pollen anaphase spindles but also the meiotic spindles. Several metaphase I cells had Y-shaped spindles which would give rise to tripolar A I divisions and could also explain the cells with misdividing normal chromosomes. HÅKANSSON (1957) reported seeing in diploid rye plants rare cases of three telophase nuclei. Another case was reported by SWAMINATHAN and NATH (1956) in *Panicum coloratum* where the presence of several accessory chromosomes at M II seemed to disturb the anaphase movements of the normal chromosomes and probably resulted in twenty per cent pollen sterility. DARLINGTON and UPCOTT (1941) noted that separate spindles in *Zea mays* were formed by the accessory chromosomes being pushed off the primary spindle. These spindles might fuse and result in tripolar spindles with irregular anaphases. A similar situation was seen by FERNANDES (1946) in *Narcissus*. BOSEMARK (1957 b) reported a change in spindle orientation caused by accessories at the second meiotic division in *Alopecurus pratensis*. Many types of abnormal A I spindles were also seen in *Tipula paludosa*

(BAUER, 1931). The degree and frequency of disturbances were directly related to the number of accessories in the spermatogonia. Unequal distribution of the chromosomes occurred; many types of spindle distortions were seen; and many variations occurred in the second meiotic division. An interaction of genes in the meiosis of *Bromus* hybrids is said to be responsible for an upset in the timing of the meiotic cycle resulting in aberrant chromosome movement (WALTERS, 1958). This article also reviews other cases of aberrant spindles which appear to be gene controlled. On the other hand, BOSEMARK (1954 a) believed that abnormalities of the first pollen mitoses with increasing number of accessories in *Festuca pratensis* might be caused by an excess of heterochromatin. LIMA-DE-FARIA (1947) reported similar abnormalities in *Anthoxanthum* like those seen in rye. He felt that these were not correlated with the accessories and that their cause could not be ascertained.

The accessory chromosomes are thought to have probably a lower proportion of active genes than ordinary chromosomes (MÜNTZING, 1945). Thus, the action of the accessory chromosomes might be considered to be merely mechanical and not caused by a genic effect or a change in the amount of heterochromatin. If the comparison is made between the number of accessories in diploids and tetraploids required to cause abnormal pollen divisions, it appears that there is no difference and thus the effect cannot be purely mechanical. One factor, however, which might influence the tetraploids is that the cell size and the resulting spindle may not be exactly doubled. The average pollen diameter in a 4n American variety of rye was increased only about seventeen per cent over diploids (MÜNTZING and PRAKKEN, 1941). However, with increasing numbers of accessories the stomatal cell sizes also increased (MÜNTZING and AKDIK, 1948 b). If this additional size is not sufficient for a normal metaphase plate to form, then the chromosomes might have to be more contracted to fit on the plate, as was found in several cells. If this is true, then the tetraploid plants may be approaching their maximum limits of tolerating extra chromosomes on a purely mechanical basis. MÜNTZING (1951 a) showed that the chromosome numbers in tetraploids could range from 26 to 31 and that 23 % of the plants were aneuploids. Although trisomics can be obtained in diploids, they show very deleterious effects from the added chromosome (MÜNTZING, 1943). He also stated that the accessory chromosome appears to have less than half the effect of an ordinary chromosome. On these bases, it would be expected that the tetraploids could tolerate more accessories than

diploids. Yet, tetraploidy does not appear to buffer the action of the accessory chromosomes. Judging from these results, the accessory chromosomes seem to have an effect which is not purely mechanical and must be genic or caused by an increase in the amount of heterochromatin.

BLACKWOOD (1956) suggested that genic control explained the behavior of accessories in *Zea mays*. Also RUTISHAUSER (1956 a) felt that since the *Trillium* fragment chromosomes had only a small amount of heterochromatin, it could be eliminated as a possible factor in causing irregular A I divisions. In the endosperms he found (1956 b) that the effect of the fragment chromosomes on breakage was not noticeably cumulative and concluded that it was genetic. The dosage effect on sperm formation in *Sorghum* was found to be geometric (DARLINGTON and THOMAS, 1941). They felt that this spindle defect leading to polymitosis was partly caused by an excess of heterochromatin. BOSEMARK (1957 a) concluded that the genetic effect of accessory chromosomes is partly governed by the genotypic constitution of the material and partly by the prevailing environmental conditions. In this rye material, wide variations were also seen between individual plants this might be attributed to different types of accessory chromosomes and effects of the internal or external environment. However, these factors probably account for only the minor variations. In rye, MÜNTZING (1949) discussed the idea that the apparent inertness of accessories may not be due to an absence of genic activity, but that the genic activities result in a balanced system. His data on fertility and vigor in structurally changed accessories, however, contradicted this idea and the data of the tetraploids also do not agree with it. It is likely that since the small accessory chromosomes were found to influence the pairing of the standard accessory chromosomes, the accessories in turn might also affect the normal chromosomes. The action of the accessory chromosome appears to delay or prevent spindle formation and seems to be genic and not merely mechanical. The per cent of abnormal cells increases with increasing numbers of accessories and reduces fertility in plants with more than three accessories.

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SUMMARY

Tetraploid plants with varying numbers of accessory chromosomes were studied at meiosis and first pollen mitosis. The frequencies, pairing and mode of transmission of the accessory chromosomes were compared with the diploid plants. The following conclusions were made:

(1) Tetraploid populations can tolerate more accessory chromosomes. Individual plants also have more accessory chromosomes since up to twelve were seen in one plant. The tetraploid population did not show an increase at the even frequencies typical of the diploids, and in the tetraploid population meiotic elimination is stronger than numerical increase owing to non-disjunction. More deviating types of accessory chromosomes were seen in the tetraploid population.

(2) Metaphase I pairing increased in both tetraploids and diploids as the number of accessory chromosomes increased, however, pairing was much poorer in the tetraploids than in the typical diploids. High multivalents were rare and univalents were more frequent than expected in plants with eight accessory chromosomes. The presence of a small iso-chromosome at M I reduced pairing of the two standard accessory chromosomes.

(3) In the other meiotic stages diploids and tetraploids behaved alike. Accessories which lagged at A I divided and were usually included in the telophase nuclei. Many A II laggards were lost as micronuclei in the tetrads and there was a large amount of elimination of the accessories. Considerable variability was seen between the plants within a group.

(4) The tetraploids compared with a diploid with a large amount of elimination appeared to have similar distributions of the accessory chromosomes at the first pollen metaphase and anaphase divisions.

(5) Metaphase and anaphase data of the first pollen division can not be used to predict the transmission of accessory chromosomes to the progeny of intercrossed plants. Diploids and tetraploids appear to have different modes of transmission of the accessory chromosomes to their progenies, however, the non-disjunction mechanism appeared to operate normally in the 4n's. These deviations can be explained by either exclusions of the accessory chromosomes showing non-disjunction from the generative nucleus or selection against gametes with many accessory chromosomes.

(6) Abnormal cells appear to be of the same types in diploids and tetraploids; however, some types seem to be related to the presence of accessories. The presence of three or more accessory chromosomes in a

pollen grain of both diploids and tetraploids caused abnormalities which were related to malfunctioning of the spindle of the first pollen division. In the meiotic divisions, tripolar spindles may also be attributed to the action of accessories.

(7) The per cent of abnormal cells in the first pollen grain divisions increased in direct proportion to the number of accessory chromosomes present. As a result, the fertility of the plants decreased as the accessories increased. The chromosome number does not appear to influence this relationship. Thus, a purely mechanical chromosomal mechanism does not seem to be the cause and the effect may be genic.

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FURTHER STUDIES ON THE BIOCHEMICAL BASIS OF PROTOPERITHECIA FORMATION IN NEUROSPORA CRASSA

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I. INTRODUCTION

IN a recent investigation of the formation of protoperithecia in *Neurospora crassa*, HIRSCH (1954) established a strong correlation between their differentiation and the formation of tyrosinase and melanin. The main points in his results were the following:

a. The dark pigment which is formed in the course of protoperithecial development is melanin, as judged from solubility characteristics, bleaching with hydrogen peroxide, and spectrophotometric investigations. It is formed only under conditions which permit protoperithecia formation, *i.e.* at a temperature below 30° C, and at low levels of nitrogen in the growth medium.

b. Under the same conditions tyrosinase activity is demonstrable in the mycelium. The activity is highest when protoperithecia formation is most intense. No formation of tyrosinase or melanin takes place if the temperature is 35° C.

c. Tyrosinase inhibitors, when used in suitable concentrations depress or suppress the formation of melanin as well as of protoperithecia. Some other enzyme inhibitors used do not show the same effect.

These results seemed to support the assumption of a close relationship between melanogenesis and protoperithecia formation so strongly as to justify further work employing this system for a study of a possible causal relation between a biochemical pathway and a pattern of differentiation. It would seem especially interesting to try to unravel the biochemistry of this pattern by the methods used in biochemical genetics for the study of growth phenomena in microorganisms (see WESTERGAARD and HIRSCH, 1954).

The utilization of these methods is, however, beset with great difficulties. Some of these are inherent in the chemical aspect of the problem

— the chemistry of melanin is far from being fully cleared up. Furthermore, most of the substances that are postulated as intermediates in the biosynthesis of melanin are very unstable (LERNER, 1953). Other difficulties are due to an insufficient knowledge of the details concerning the morphology and the differentiation of protoperithecia.

Before a systematic investigation can be started, using the usual biochemical-genetical methods, it appears desirable to test further the theory of a relation between melanogenesis and protoperithecia formation, especially as concerns the following points:

1. To investigate whether the absence of tyrosinase activity that is characteristic of normal mycelia at 35° C is found in genetically sterile mutants at 25° C, under conditions normally permitting the formation of protoperithecia.
2. To determine, if possible, the relationship between phenylalanine and tyrosine content of the mycelia on one hand, and environmentally and genetically controlled sterility, on the other.

Some details of the results here presented were published in preliminary form by WESTERGAARD and HIRSCH (1954).

II. MATERIAL AND METHODS

The *Neurospora crassa* strains used were the following:

W 2/49. This is a colonial, biochemically wild type, strain selected for its high fertility from a cross between an adenineless colonial strain *W 40A* and the wild type strain *Emerson 5257a* (KÖLMARK and WESTERGAARD, 1953). It is the strain used by HIRSCH (1954).

Sterile mutants *913/83*, *93/1*, and *143/33*. They are melanin free, non-protoperithecia forming strains selected from crosses between different female sterile *Neurospora* strains.

Strain *1211/31* was selected from the same crosses; it is characterized by excessive melanin formation in combination with large numbers of somewhat abnormal protoperithecia.

The culture medium was the protoperithecia-promoting medium (p-minimal) of WESTERGAARD and MITCHELL (1947). It was used in liquid form. To each petri dish were added 15 ml of the substrate and 5 sheets of sterile Whatman no. 4 filter paper, 8 cm. Cultivation on filter paper was necessary, as *W 2/49* does not form melanin or protoperithecia on liquid medium alone. Inoculation was done with 0.5 ml of a suspension of conidiaspores in sterile distilled water. The mycelia were

harvested from the Petri dishes, rinsed with distilled water, and dried. Details of the experimental procedures will be given in the different sections.

III. RESULTS

1. Tyrosinase activity

The procedure used resembles that published by HIRSCH (1954). The mycelium was harvested from the Petri dishes, rinsed with redistilled water, dried with filter paper and weighed. It was homogenized in an ice-cold mortar with ice-cold M/10 phosphate buffer, pH 6.0, without addition of abrasives. 1 ml buffer was used for 50 mg mycelium. The mycelium was centrifuged at 3000 rpm and 0.5 ml of the supernatant added in a colorimeter tube to 5 ml buffer and 1.5 ml tyrosine (300 mg in 100 ml of M/60 NaOH). Blanks contained buffer instead of tyrosine. The tubes were placed in a constant temperature water bath at 30° C, and the activity measured at intervals of 15 minutes in an EEL colorimeter with blue filter No. 303. Dialysis was done against cold M/60 phosphate buffer, pH 6.0, either with mechanical stirring for about 2 hours, or without stirring for 24 hours.

HIRSCH (1954) measured tyrosinase activity and protoperithecia formation as a function of age in strain W 2/49, when grown at 25° C. No tyrosinase activity could be demonstrated in this strain when grown at 35° C. At this temperature the mycelium contains no melanin and forms no protoperithecia.

In the present experiments measurements of tyrosinase activity were made on the female sterile strains 913/83, 143/33, and 93/1, all melaninless, and on the high-melanin strain 1211/31. In the case of the latter, an activity somewhat lower than that of W 2/49 was found. None of the three strains without protoperithecia showed any tyrosinase

TABLE 1. *Tyrosinase activity found in undialysed mycelial extracts of the five strains: W 2/49, 1211/31, 913/83, 143/33, and 93/1, grown for 6 days at 25° C.*

The figures show optical density multiplied by 100 (Zero time readings *not* deducted).

Minutes	W 2/49	1211/31	913/83	143/33	93/1
15	4.2	1.2	0.0	0.0	0.7
30	11.4	1.8	0.0	0.0	0.7
45	22.4	3.6	0.0	0.0	0.2
60	34.4	7.8	0.0	0.0	0.8
75	44.7	13.2	0.0	0.0	0.6
90	54.0	18.6	0.0	0.0	0.8

activity, with or without dialysis. The activity found in the four strains after 6 days' growth at 25° C. is shown in Table 1, together with the corresponding measurements of activity in mycelia of W 2/49.

2. Chromatographic determination of aromatic amino acids

Paper chromatography. The solvent systems employed were (CRAMER, 1953):

I. Secondary butanol-formic acid-water, (75 : 15 : 10).
II. Phenol-water, (4 : 1), to which was added 0.04 % 8-oxychinolin.

The chromatograms were run at 23.5±0.2° C, in the first direction for about 20 hours, in the second for about 24 hours. Whatman no. 1 filter paper was used throughout. The ascending technique was employed. The paper was kept in the chromatographic chamber for some hours to obtain equilibration with the atmosphere, and then dipped into the solvent. The chromatograms were dried overnight at room temperature and sprayed with a 0.2 per cent solution of the modified ninhydrin reagent of LEVY and CHUNG (1953). The chromatograms were not heated, the reagent was allowed to act at room temperature, and the result could be read after a few hours.

Working procedure. The mycelia were harvested on day 3, 5, 7, 10, and 14—15. In a few instances the growth of a mutant was so poor after 3 days that harvesting was impossible. The investigation was then carried out on mycelia 5, 7, 10, and 15 days old. The mycelium was rinsed with distilled water and dried with filter paper. An aliquot was taken out and placed in a desiccator with $CaCl_2$ for determination of dry weight. The rest of the mycelium was used for a determination of the content of aromatic amino acids, which were separated from the aliphatic amino acids by adsorption on carbon and selective elution. (SCHRAMM & PRIMOSIGH, 1943).

The separation was carried out on a column, about 5 cm high, of Merck's pulverized carbon p.a. (about 4 grams). To avoid irreversible adsorption, the addition of mycelial extract had to take place in 5 per cent acetic acid. The mycelium was, therefore, homogenised in a mortar with 5 per cent acetic acid. The ground mycelium was boiled in a water bath for a few minutes to precipitate the proteins, and then centrifuged at 3000 rpm for about two minutes.

Before adding the extract the column was rinsed for some hours with 5 per cent acetic acid. After adsorption on carbon the aliphatic

amino acids were eluted with 75 ml of 5 per cent acetic acid. The aromatic amino acids were then eluted by displacement with 100 ml 5 per cent phenol in 20 per cent acetic acid.

The solution of aromatic amino acids was evaporated in vacuo, water was then added, and the evaporation repeated several times to remove the phenol. After evaporation to dryness a few ml of water were added.

Equivalent amounts of the solutions of aromatic amino acids from the different mycelia were placed on the paper chromatograms. The chromatograms were run either two dimensionally in both solvents or in one dimension in either the butanol solvent or the phenol solvent. In the last case solutions from the same strain but of different ages were placed on one chromatogram, so that it was possible to compare directly the content of aromatic amino acids in mycelia of different ages. Synthetic amino acids were run as controls on the same chromatogram. On all chromatograms glycine was used as a marker.

Results. Only phenylalanine and tyrosine were detected. Tryptophane was destroyed by the precipitation of protein, and the presence of Dopa (3,4 dihydroxy-phenylalanine) could not be demonstrated with certainty. The amount of phenylalanine and tyrosine was not determined by quantitative methods, as visul inspection clearly showed the results.

The 25° mycelium of strain W 2/49 shows a very sharp fall in the amount of the two amino acids between the third and the fifth day, when the differentiation of protoperithecia as well as the formation of melanin begins. In contrast to this, the mycelium grown at 35° C shows a quite gradual decrease in the content of these two amino acids which, on the fifth day, are present in nearly as high a concentration as that in mycelium harvested on the third day. This is in accordance with the fact that neither protoperithecia nor melanin are formed at 35° C.

Strain 1211/31, which is characterized by excessive melanin and protoperithecia formation, shows the same picture as the 25° mycelium of W 2/49, but with an even sharper decline between the third and fifth day. The protoperithecia free "albino" strains 913/83, 143/33, and 93/1 show the same picture as the 35° mycelium of W 2/49, *i.e.* a high content of phenylalanine and tyrosine even after the third day, and a gradual decrease with increasing age.

DISCUSSION

The results of the present investigation may be summarized as follows:

No tyrosinase activity is demonstrated in mutants of *Neurospora crassa*, which, by visual inspection, are selected as being melanin free and non-protoperithecia forming (female sterile) when these mutants are grown at 25° C. Consequently these mutants behave at 25° C, as the female fertile stains behave at 35° C. Tyrosinase is, however, present in the high-melanin strain 1211/31 at 25° C. This strain shows a tyrosinase activity a little lower than the normal strain which suggests that the limiting factor in the formation of melanin and protoperithecia is the substrate, not the enzyme.

This is born out by the chromatographic investigations. The content of tyrosine and phenylalanine is much greater in the high-melanin strain than in normal mycelia of the same age. An accelerated decline in the amount of these amino acids is observed when the excessive formation of abnormal protoperithecia and melanin sets in. This strain thus appears as an extreme variant of the normal sexual strain, also with respect to the content of tyrosine and phenylalanine. On the other hand, the asexual strains show the same picture as melanin-free sterile 35° C mycelia of the normal strain, *i.e.* a high content of tyrosine even after the time when it has practically disappeared in the sexual strains, due, undoubtedly, to its utilization as the substrate for tyrosinase.

The content of tyrosinase and aromatic amino acids in normal *Neurospora crassa* as well in environmentally and genetically sterile mycelia therefore seem correlated with the formation of protoperithecia in a way that would be expected if the differentiation of these structures were dependent on one or more substances formed by the action of tyrosinase on its substrate. The present results are, therefore, completely consistent with the idea put forward by WESTERGAARD and HIRSCH, that in the formation of the female sex organs in *Neurospora* one or more of the precursors of melanin may have a hormone-like effect.

The possibility of the existence of a correlation between melanin and sexual differentiation has been investigated in other fungi. CANTINO studied the biochemical basis of the formation of the socalled resistant sporangia in *Blastocladiella emersonii* (for review see CANTINO 1956). This fungus produces two sorts of sporangia: thinwalled, unpigmented zoosporangia and thickwalled, melanin incrusted resting bodies, "resistant sporangia" (RS), which, from analogy with *Allomyces*, are assumed to be meiotangia. CANTINO showed that a polyphenol oxidase is present in RS-plants whereas it is absent in plants that form thinwalled sporangia. He found it possible, however, to suppress the formation of melanin with phenylthiourea and still get "structurally-normal

and perfectly-viable" RS. As the primary biochemical basis for the differentiation of RS he postulates a "triggermechanism" with bicarbonate as the initiating factor and α-ketoglutarate oxidase as the focus for its action. The normal biosynthesis of melanin is coupled to this system in such a way that α-ketoglutarate is found to be a necessary cofactor for the action of the polyphenol oxidase *in vitro*; dialysis of the homogenate of RS-plants removes allmost all activity, which is regained when α-ketoglutarate is added to the reaction mixture.

Such a system does not seem to exist in *Neurospora*. Dialysis in our strains has no effect on tyrosinase activity, and HOROWITZ and SHEN (1950) found in some cases dialysis a necessary prerequisite for the demonstration of tyrosinase activity. Also, the reaction to tyrosinase inhibitors suggests another mechanism, as HIRSCH (1954) found that phenylthiourea caused suppression of both tyrosinase and protoperithecia formation in *Neurospora crassa*.

ESSER (1956) studied growth, fructification, and pigmentation in *Podospora anserina* on different synthetic media. He found, on visual inspection, no correlation between the differentiation of protoperithecia and the formation of a pigment which he assumed to be melanin, either in the wild type or in different mutants of *Podospora anserina*. Tyrosinase activity was not investigated.

KUWANA (1956) examined different strains of *Neurospora crassa* for tyrosinase activity, originally with the intention of clarifying the biochemical differences between mating types A and a. He found an increased tyrosinase activity in mixed cultures A+a as compared with that in A and a mycelia cultivated separately, and only under conditions where the A+a culture was allowed to form abundant perithecia. Later he found, however, that the genetic factors for increased tyrosinase activity segregated from the mating type locus, and further that he got the same increased activity in some A+A and a+a cultures. On the basis of his experimental results he excluded the possibility that the increased activity in the mixed cultures might be due to a destruction of or a failure to synthesise inhibitors. He regards the increased formation of tyrosinase in the mixed cultures as a real phenomenon, but concludes that the cause of the increase still remains to be determined. Nothing in the results seems to exclude an explanation which assumes the formation of heterocaryons accompanied by disappearance of the mutant character due to the fact that different loci involving tyrosinase activity are present in the two strains. Tyrosinase activity was low or

absent in all single cultures A or a. Absence of activity in mixed cultures would then suggest that the responsible genes in these two strains were allelic.

In a later publication (KUWANA, 1958) it is concluded from heterocaryon experiments that the growing hyphae are composed of only one kind of nucleus. No information is, however, given about melanization of the mycelia in the heterocaryon experiments.

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SUMMARY

The experiments reported in this paper add further biochemical confirmation to the hypothesis that a close relationship exists between melanin formation and development of protoperithecia in *Neurospora crassa*.

Tyrosinase activity and the content of free phenylalanine and tyrosine were investigated in three female-sterile strains and in a high-melanin strain, which forms a very large number of somewhat abnormal protoperithecia.

In both respects the female-sterile strains behave as environmentally sterile mycelia of the normal wild type. The high-melanin strain appears as an extreme variant of the normal fertile wild type.

The results are discussed in relation to other data concerning melanin and sexual differentiation in fungi.

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THE VEGETATIVE NUCLEI OF THE FUNGUS OPHIOSTOMA

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INTRODUCTION

THE ascomycete *Ophiostoma multiannulatum* has been the object of morphological studies at this institute during the last few years (A. and B. v. HOFSTEN, 1958). Recent experiments concerning spontaneous and induced backmutations have also been published (ZETTERBERG and FRIES, 1958). During the course of this work it became desirable to know more about the number of nuclei in different types of cells and their mode of division.

The life cycle of *Ophiostoma* (*Ceratostomella*) has been described by ANDRUS (1936), but he chiefly studied the formation of the perithecia and did not describe the distribution of nuclei in the vegetative cells. The classic study on *Neurospora* by MCCLINTOCK (1945) has shown that it is possible to use conventional cytological technique for studies of the nuclei in fungi. The recent work of ROBINOW (1957) suggests that the mitotic process in fungi may be different from that of higher plants. In the present paper a staining technique useful for the mitotic nuclei of *Ophiostoma* will be described. The distribution of nuclei in various types of cells will also be presented, but a more detailed investigation on the actual process of mitosis in cells under normal and growth inhibiting conditions is in progress.

MATERIALS AND METHODS

Cultures of *Ophiostoma* were maintained by regular transfers on agar and in liquid media of compositions described in an earlier paper (A. and B. v. HOFSTEN, 1958). In the experiments with *Ophiostoma multiannulatum*, a typical wild strain was used, but certain comparative experiments were also performed with some biochemical mutants of the same organism, and with the closely related species *Ophiostoma piceae*.

Preliminary staining experiments with haematoxylin gave very unreliable results. After experiments with the Feulgen technique, a modification of the staining procedure using azure A, described by HUEBSCHMAN (1952), was found to give the best results.

The cell material was fixed and stained either on albumen-covered slides or directly on small pieces of cellophane as described by ROBINOW (1957). After fixation for 25 minutes in alcohol-glacial acetic acid in the proportion 3 : 1, the cells were transferred to 1 N HCl at 60° for hydrolysis. After a brief wash in water, the cells were stained for twenty-five minutes in a solution containing:

Azure A (Gurr)	2 g
1 N HCl	6 ml
10 % $NaHSO_4$ [1]	6 ml
distilled water	100 ml

By this procedure, the nuclei were stained dark blue in wet mounts. For permanent mounts, the cells were washed in alcohol and xylene and mounted in Clarite (The Neville Co.).

With the fixative employed, the size and shape of conidia did not change appreciably unless they were dehydrated too violently. For young hyphae, a different fixative containing six parts absolute alcohol, one part glacial acetic acid and one part lactic acid, as used by SINGLETON (1953), gave better results. Osmic vapour and fixatives containing mercury were less satisfactory. At room temperature, the fixed material can be kept only during one day without deformation occurring, but at +4°, it may be preserved for months.

The optimal time for hydrolysis was found to vary with the type of cell material. Conidia and ascospores require about ten minutes, but vegetative hyphae do not tolerate more than five minutes. When hydrolysis is completely omitted, no nuclei can be distinguished in the cells.

Attempts to find a specific stain for the cell wall of *Ophiostoma* have been unsuccessful. A solution of 0.5 % eosin in 95 % alcohol will stain the cytoplasm red. This increased the contrast and facilitated the detection of the boundaries between the individual cells. Distinct crosswalls could also be seen when the preparations were observed in the microscope under phase contrast illumination.

Microscopic observations were made with a Zeiss Opton microscope equipped with phase contrast, a lens 100x (N. A. 1.25), oil between top

[1] not $NaHSO_4$ as erroneously stated in HUEBSCHMAN's (1952) description!

slide and lens, and 8x oculars. The microphotographs were taken on Ilford F.P. special plates, or on Adox KB 14, 35 mm film, using the same microscope and a green filter.

RESULTS

Ophiostoma may form asexual conidia from the tips of hyphae when grown on a solid medium. In a synthetic liquid medium, these conidia are able to multiply by a budding-process. On solid media, or in certain liquid media, they grow out into hyphal cells. The ability to grow as a homogeneous suspension of budding conidia varies with the genotype, with the temperature of incubation and with composition of the medium. The various physiological factors which influence the vegetative morphogenesis of *Ophiostoma* have been discussed in a previous paper (A. and B. v. HOFSTEN, 1958).

When a homogeneous suspension of resting conidia of *O. multiannulatum* is stained with azure A, only *one* nucleus can be detected in each cell. The nuclei in the cells illustrated in Fig. 1 appear as densely stained, blue bodies in good contrast to the surrounding cytoplasm. If the conidia are grown at a temperature of 20° or lower, the conidia have a more heterogeneous appearance. Thus, many of the germinated conidia in Fig. 2 have more than one nucleus. This is because they are septated, and crosswalls can actually be seen in most such cells.

The actual mechanism of nuclear division in *Ophiostoma* is very difficult to study in detail owing to the small size of the chromatinic elements. When ordinary dividing conidia are stained with azure A, one can only distinguish a dense nucleus in the cells. However, if such cells are kept at +4° overnight before staining, the nuclei appear less dense, and it is possible to distinguish chromatinic elements separated from each other (Fig. 3). It is tempting to count the number of chromosomes in such preparations but due to the small size of the nuclei, the present estimations require confirmation from further studies of the meiotic division. No mitotic apparatus has been observed, and no typical metaphase stages in equatorial arrangements have been distinguished. Stages equivalent to the anaphase are more easily found as shown in Fig. 4.

When *Ophiostoma* is grown in liquid cultures in the presence of 0.4 % yeast extract, most conidia will grow out into long septate hyphae. Fig. 5 shows such cells, and in each hyphal segment only one nucleus can be detected. On the other hand, under conditions, where growth is more

or less inhibited *Ophiostoma* will form many filamentous unseptate cells. The nuclei in such cells are very difficult to stain.

With another species, *Ophiostoma piceae*, some comparative experiments have been made. Fig. 6 shows conidia and septate hyphae grown in a synthetic liquid medium, and there is only one distinct nucleus in each hyphal cell.

Ophiostoma is a heterothallic fungus and when strains of opposite mating type are cultivated together on 0.2 % malt extract media, asci will develop in special perithecia. Each ascus contains eight ascospores, but it is not possible to isolate them one after the other as in *Neurospora*. Fig. 7 shows a young ascus with eight spores in a circular arrangement. A group of mature ascospores, leaving the perithecium in a viscous drop of solution is seen in Fig. 8, and each spore contains only one nucleus.

DISCUSSION

Various methods have been used for the staining of mitotic nuclei in fungi. ROBINOW (1957) has compared several different stains and found that haematoxylin is unsuitable for the staining of the chromatinic elements in the lower fungi. In *Mucor*, *Phycomyces* and *Blastomyces* only the nucleolus is stained with haematoxylin according, to ROBINOW (1957) and BAKERSPIEGEL (1957), and very little can be said about the nuclear division when this stain is used. A simple staining procedure with orcein has been found to give very good results in *Neurospora* by MCCLINTOCK (1945) and SINGLETON (1953). With *Ophiostoma*, however, hydrolysis before the staining seems to be necessary in order to destroy all stainable elements in the cytoplasm.

Azure A has long been used as a nuclear stain and seems to be of special value for the staining of microorganisms. Excellent results have been obtained with bacteria (DE LAMATER, 1951), in *Neurospora* (HUEBSCHMAN, 1952), and in several Phycomycetes (ROBINOW, 1957, BAKERSPIEGEL, 1957). The present author has found that nuclei in both the conidia and the hyphae of *Ophiostoma* are stained much better with azure A than with the basic fuchsin in the Feulgen procedure.

Both hyphae and spores of the Phycomycetes and many Ascomycetes, are usually multinucleate. ROBINOW (1957) demonstrated spores of *Phycomyces* with up to six or seven nuclei. BAKERSPIEGEL (1957) has pictures of hyphae in *Blastomyces* with up to twelve nuclei, and some of them seem to be able to migrate through pores in the crosswalls. *Eremascus* (DE LAMATER, 1953) has several nuclei in the cells of young

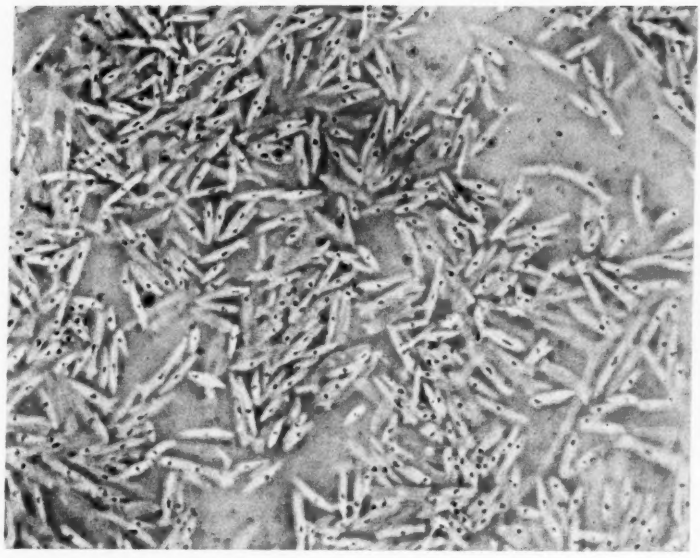
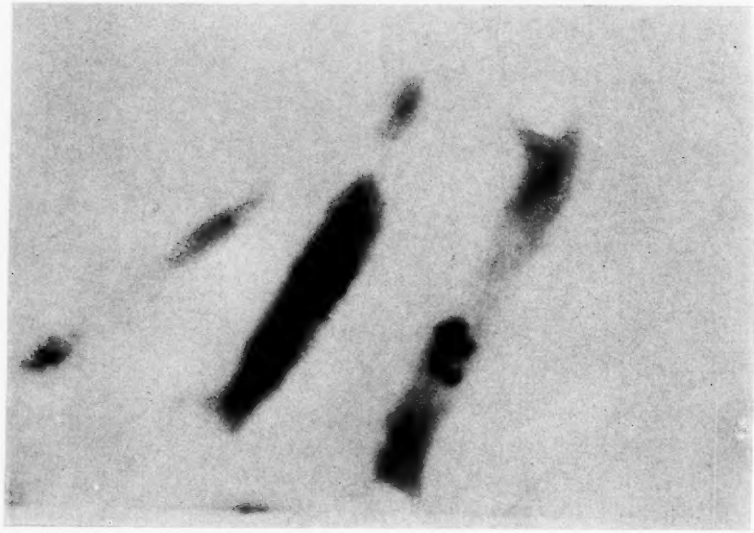


Fig. 1. Uninucleate conidia of *Ophiostoma multiannulatum* stained with azure A after hydrolysis. — Fig. 2. Nuclei in septated, germinating conidia of *Ophiostoma multiannulatum*. Azure A after hydrolysis. — Fig. 1, ×500; Fig. 2, ×3000.



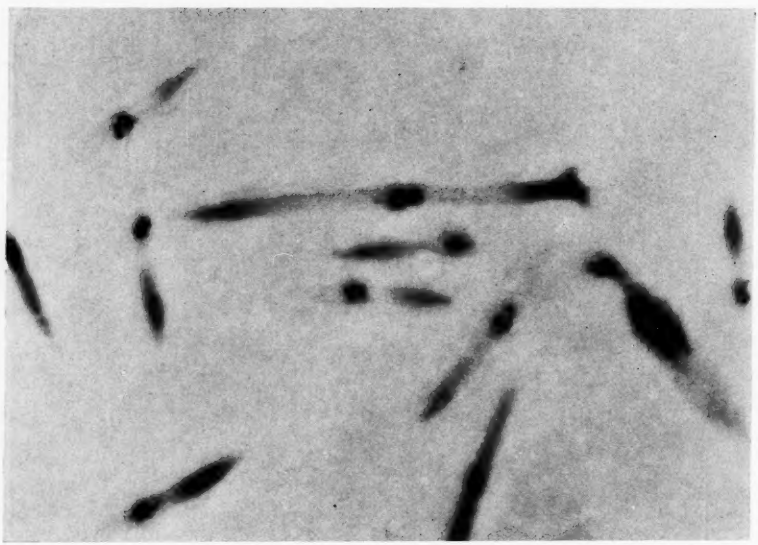


Fig. 3. Chromosomal structures in conidia of *Ophiostoma multiannulatum* stained with azure A and eosin after cold treatment and hydrolysis. — Fig. 4. Dividing nuclei in conidia of *Ophiostoma multiannulatum*. Azure A and eosin after cold treatment and hydrolysis. — Fig. 3, ×5000; Fig. 4, ×3000.

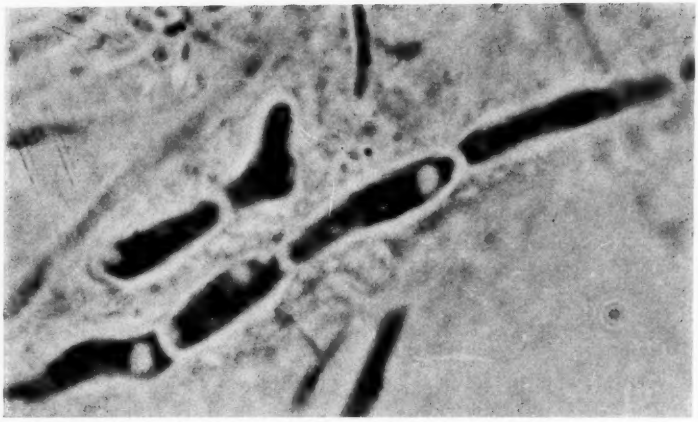
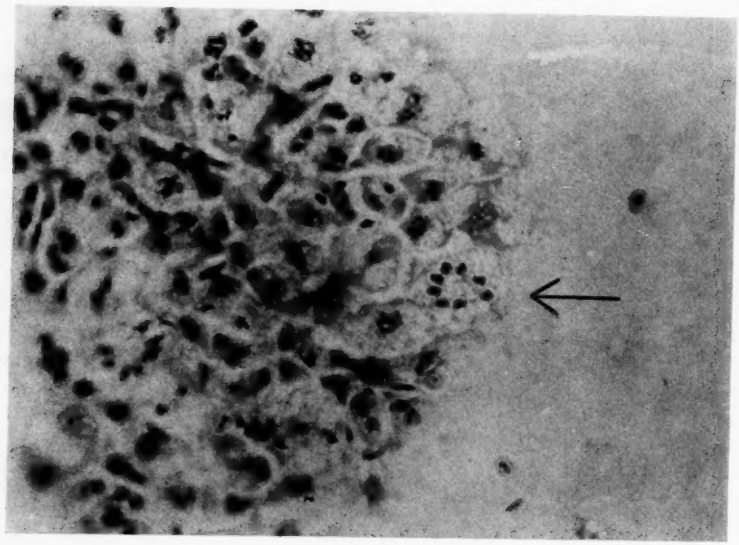


Fig. 5. Nuclei of septated hyphae of *Ophiostoma multiannulatum* grown in a medium containing yeast extract. Azure A and eosin after hydrolysis. — Fig. 6. Uninucleate cells of *Ophiostoma piceae*. Azure A after hydrolysis. — Fig. 5, ×3000; Fig. 6, ×1500.



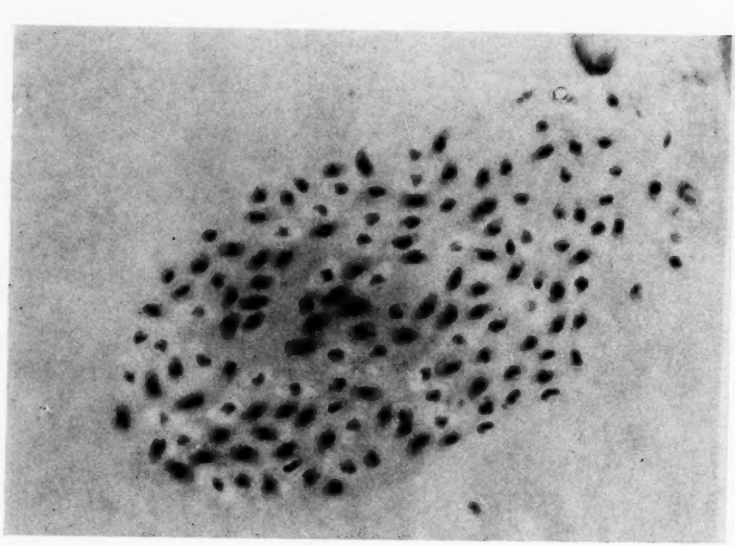


Fig. 7. Various stages in the formation of asci of *Ophiostoma multiannulatum*. The arrow indicates an ascus with eight spores. Azure A and eosin after hydrolysis. — **Fig. 8.** Ascospores of *Ophiostoma multiannulatum*. Azure A and eosin after hydrolysis. — Figs. 7—8, ×2500.

rapidly growing hyphae, and in *Neurospora* (SINGLETON, 1953) the ascospores are binucleate, while the hyphal cells and conidia are uni- to multinucleate. However, in *Dipodascus uninucleatus* BIGG (1937) has shown that the cells are consistently uninucleate.

As shown in this investigation all cells of *Ophiostoma multiannulatum* are uninucleate at least if they are allowed to grow in media permitting normal growth. Even the ascospores leave the perithecium in a uninucleate stage. HUEBSCHMAN (1952) has found that in *Neurospora* the number of nuclei in the conidia varies with the composition of the growth medium. The addition of yeast extract and amino acids to a minimal medium will increase the number of nuclei per conidium from an average of 2.6 to 6.2. In *Ophiostoma*, growth in a complex medium only results in a greater proportion of hyphae, but all single cells are still uninucleate.

Heterocaryosis is a phenomenon that has been studied in several fungi. It has been found not only in heterothallic fungi, but also in many homothallic species (OLIVE, 1953). An essential requirement for the production of heterocaryotic mycelia is that the cells contain more than one nucleus. FRIES and TROLLE (1947) have unsuccessfully tried to produce heterocaryotic mycelia from different physiological mutants of *Ophiostoma*. As was also assumed by these authors, the explanation for this failure is that the normal cells of this organism are normally uninucleate, which excludes heterocaryosis.

In a previous paper about the morphogenesis in *Ophiostoma* (A. and B. v. HOFSTEN, 1958), it was shown that exponentially growing conidia may be grouped into several distinct size groups. The possibility that this reflected a variation in the number of nuclei per cell can now be eliminated.

Acknowledgement. — The author is much indebted to Professor NILS FRIES and Docent BENGT KILHMAN for helpful criticism and stimulating discussions.

SUMMARY

(1) The nuclei of the ascomycete *Ophiostoma multiannulatum* have been stained with azure A after hydrolysis in HCl.

(2) The number of nuclei in different types of cells has been estimated. Conidia grown in a liquid medium have only one nucleus. In septate hyphae obtained in the presence of 0.4 % yeast extract, only one nucleus can be detected in each hyphal cell.

(3) When strains of opposite mating types are cultivated together, asci will develop with eight ascospores. Each ascospore has only one nucleus.

(4) Some comparative experiments have been made with *Ophiostoma piceae*, and only one distinct nucleus in each cell can be demonstrated in this species.

(5) The cytological results obtained by use of azure A are discussed in relation to similar investigations in other microorganisms. Fruitless attempts to produce heterocaryotic mycelia in *Ophiostoma* can be explained by the consistent uninucleate stage of the cells.

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CHANGE OF LENGTH AND VOLUME OF MITOTIC CHROMOSOMES IN LIVING CELLS

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I. INTRODUCTION

IN studies on chromosome structure special attention has been drawn to the spiralization cycle of meiotic chromosomes and, to a smaller extent, of mitotic ones. Important reviews on the subject were published by: NEBEL (1939), KUWADA (1939), KAUFMANN (1948), MANTON (1950) and others. Although the studies have shown the existence of the spiral structure of the chromosomes in meiosis and mitosis, the details and especially the mechanism of coiling are incompletely known.

Some other aspects of the chromosome changes were studied by a few authors only. It is known from the time of FLEMMING (1882) that the chromosomes are considerably shorter in metaphase than in prophase, but only a few numerical data concerning the changes of length are available. Also, it is not generally known at what stage of mitosis the chromosomes are shortest, and very often the view of DARLINGTON that "in most organisms contraction of the chromosomes reaches its maximum at the last stages of prophase" (DARLINGTON 1937 p. 25) is held. BĚLAŘ (1929), however, has pointed out that the chromosomes contract very strongly and rapidly during anaphase and telophase. Important data concerning the chromosome length are to be found in the works of BELLING (1928), MANTON (1935, 1939, 1945, 1949), SPARROW et al. (1941), SVÄRDSON (1945) and WICKBOM (1949). BELLING (1928) and MANTON (1935) studied also the changes in volume of the chromosomes. The difference in chromosome length between normal mitosis and colchicine mitosis was measured by ÖSTERGREN (1944).

All the measurements described in the above papers, except those of BĚLAŘ (1929), were made on fixed material. Only very fragmentary data

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concerning the changes of living chromosomes are available (viz. those from BĚLAŘ 1929). For this reason the present author decided to study the changes in chromosome length and volume by means of measurements made on the films of mitosis in the endosperm of *Haemanthus katharinae* BAK. and *Leucojum aestivum* L. (BAJER 1957, MOLÈ-BAJER 1958). The data concern the period from prophase before the nuclear membrane disappears until the formation of the cell plate in telophase.

II. MATERIAL AND METHODS

The measurements were done only on very flattened cells of *Haemanthus katharinae* and *Leucojum aestivum* in which the chromosomes were arranged in a single layer. The method of flattening the living endosperm cells was described previously (BAJER 1955, 1957, BAJER and MOLÈ-BAJER 1956). Time lapse cinemicrography with both 35 mm and 16 mm film was used and measurements were done on enlarged prints (cells Nos. 405, 207, 281) and on drawings (cells Nos. 157, 99). The measurements on *Haemanthus* were done on 3 cells undergoing normal mitosis and on 1 cell dividing under the influence of colchicine (the influence of colchicine on the course of mitosis in living cells of endosperm was described by MOLÈ-BAJER 1958). Not even in these flattened cells could the changes in length of the whole triploid chromosome set (27 chromosomes) be measured. However, the length of no less than 17 chromosomes was measured in three cells and the length of 24 in two other cells. The change of chromosome length was also measured in one cell of *Leucojum aestivum* (Fig. 9).

During the measurements the film was projected many times to avoid errors in the estimation of the position of kinetochores and chromosome ends. This is of particular importance during prophase and prometaphase.

The volume of the chromatids from prophase until the beginning of anaphase and of the daughter chromosomes in anaphase, was calculated from measurements of the length and the breadth of the chromatids and daughter choromosomes on the assumption that the cross section of these bodies is a circle. The same assumption was previously made by BELLING (1928), MANTON (1935) and SVÄRDSON (1945).

III. RESULTS

1. Chromosome morphology

The endosperm of *Haemanthus katharinae* is a triploid tissue 3n=27. In the haploid chromosome set there are 4 large chromosomes and

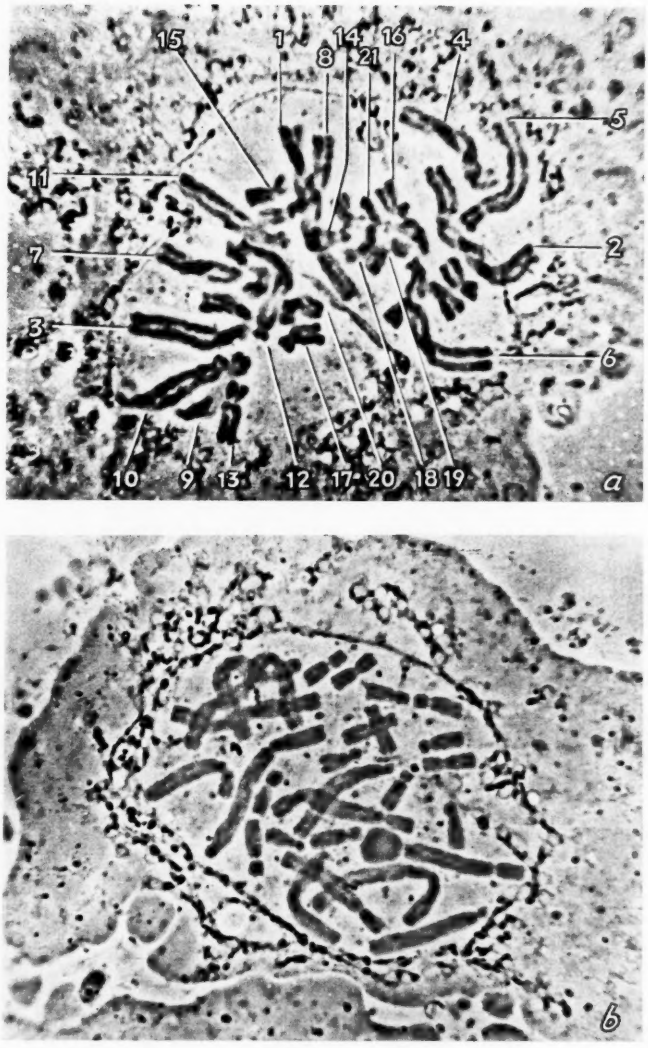

Fig. 1. Flattened cells of *Haemanthus katharinae* in the living state. a. Cell (No 405/55) on which the measurements of chromosome length (Figs. 5—6) and volume (Fig. 8) were made. Nos. of chromosomes given in micrograph. b. Cell under the influence of 50 ppm. of colchicine in agar. Micrograph from the work of MOLÈ-BAJER (1958). No measurements were made on this cell.

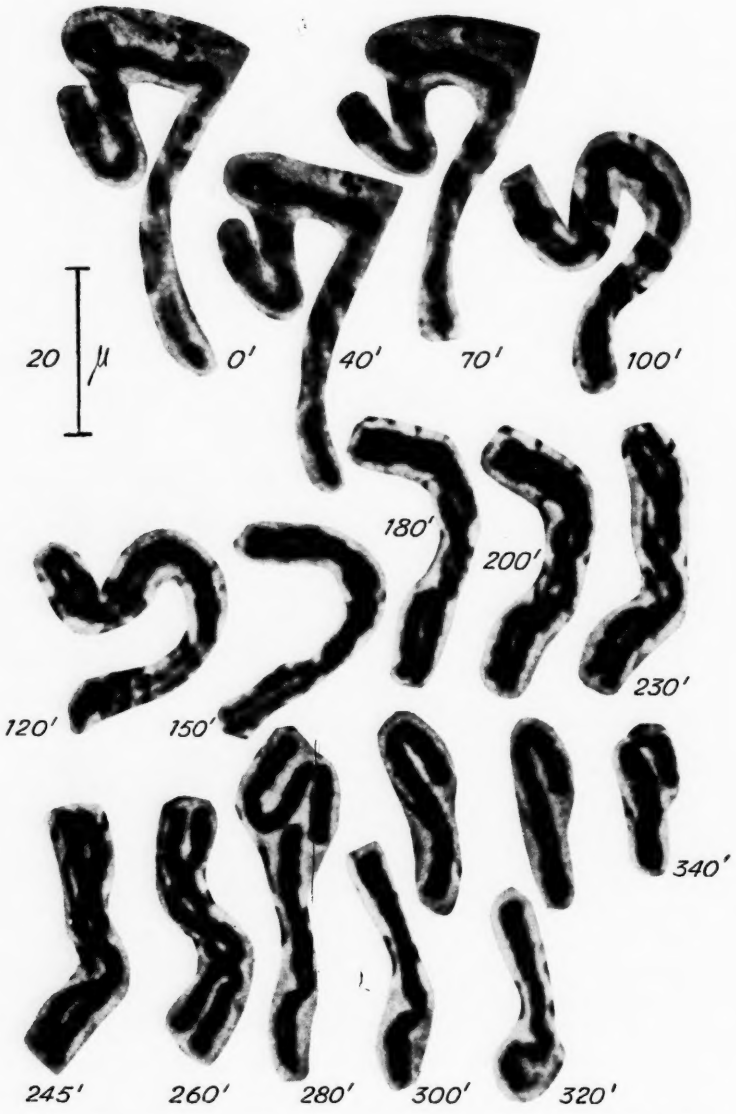

Fig. 2. Chromosome No 2 from the cell of Fig. 1 a; in Fig. 6=black circles.

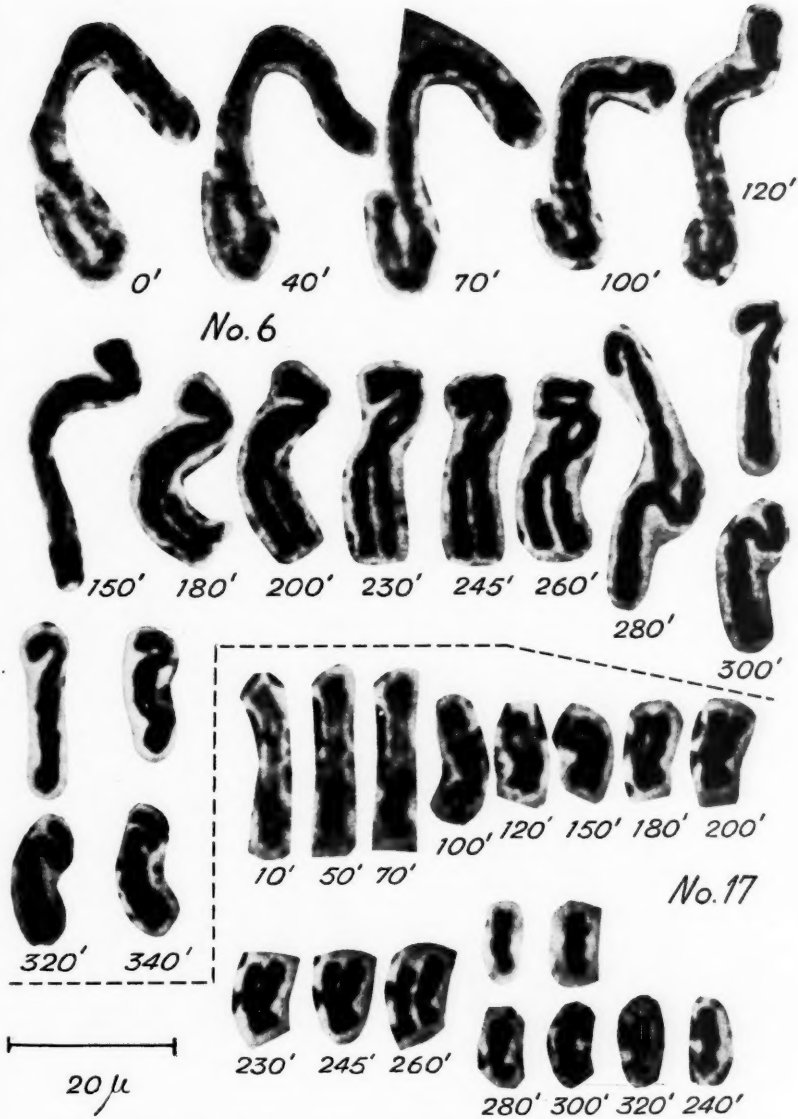

Fig. 3. Chromosome No 6 from the cell of Fig. 1 a; in Fig. 5=black triangles. Also small chromosome No 17; in Fig. 6=black circles.

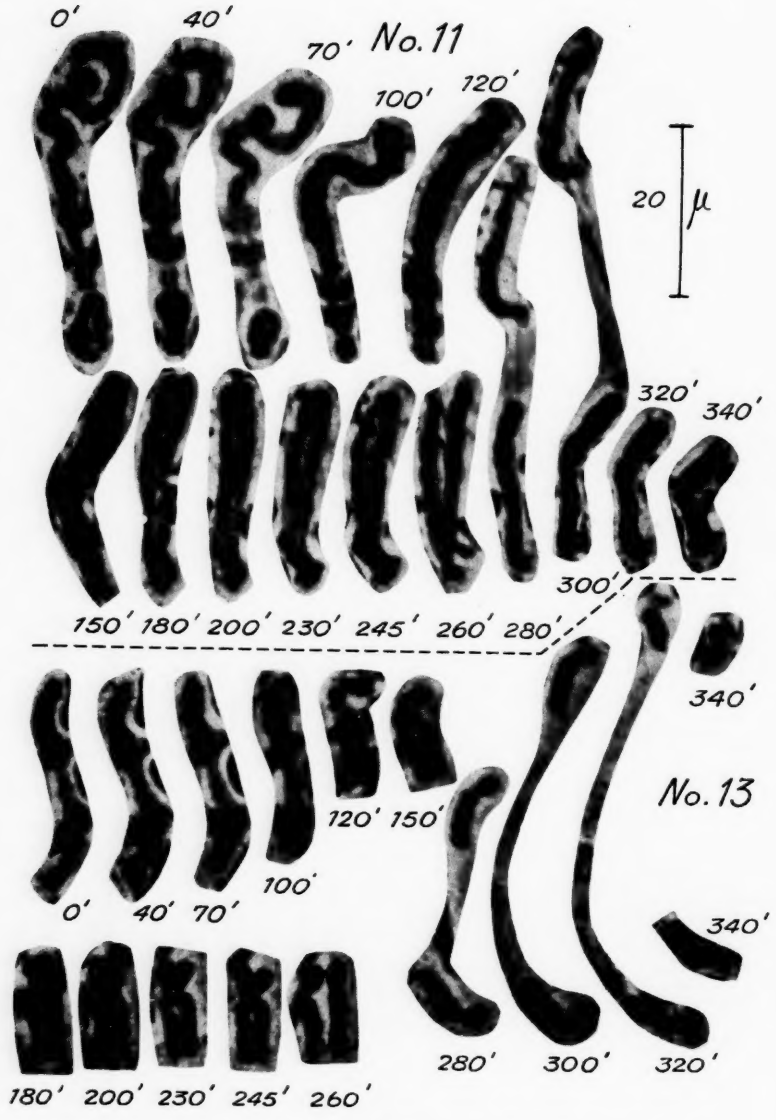

Fig. 4. Chromosomes Nos. 11 and 13 from the cell of Fig. 1 a; in Fig. 6 =black triangles and white circles respectively.

5 smaller ones. Each of the four large types may be easily distinguished in the living state: they differ in their dimensions and in the position of their kinetochores. At metaphase the arm ratio of the largest chromosome type is approximately 1 : 2 (Figs. 1—2, chromosomes 1, 2 and 3) the second largest type is slightly smaller and has the arm ratio of approximately 1 : 3 (Fig. 1, chromosomes 4, 5, 6 and Fig. 3), the third type is smaller still and its arm ratio is also approximately 1 : 3 (Fig. 1, chromosomes 7, 8 and 9) and the fourth type is approximately of the same size as the third, but it has a subterminal kinetochore (Fig. 1, chromosomes 10, 11 and 12 and Fig. 4). Among the smaller chromosomes at least three types may be distinguished; one chromosome type (Fig. 1, chromosome 13 and Fig. 4) is slightly larger than the others. The remaining two differ in the location of their kinetochores. Because of their small dimensions and the possibility of mistakes in identifying them, no efforts were made to distinguish the homologous types within this latter group.

Chromosome morphology in *Leucojum* was not studied (see however, micrograph of chromosome set in living state, BAJER 1955).

2. Changes in chromosome length

Similar changes were found in all chromosomes in all cells of *Haemanthus* on which the measurements were made. The numerical data were slightly different in different cells during normal mitosis, and the chromosomes were more strongly contracted during colchicine mitosis.

In normal mitosis (Figs. 5—6) the chromosomes start a period of rapid shortening a few minutes before the dissolution of the nuclear membrane. They shorten most rapidly during prometaphase at the same time as the chromosomes execute their most vigorous movements before the formation of the metaphase plate. This is similar to the results of SVÄRDSON (1945) on animal material; he found that "the shortening is more marked between pro- and mid-metaphase than between mid- and late metaphase" (l. c. p. 24). At this stage in *Haemanthus* the largest chromosome may shorten at the ratio of 0.7 μ/min. Gradually the chromosomes shorten more slowly and there is a short time in metaphase when there is almost no change in the large chromosomes and in the smaller ones the change is not measurable. Just before the start of anaphase the chromosomes begin to shorten again and they continue their shortening during the whole of anaphase. This result of the present

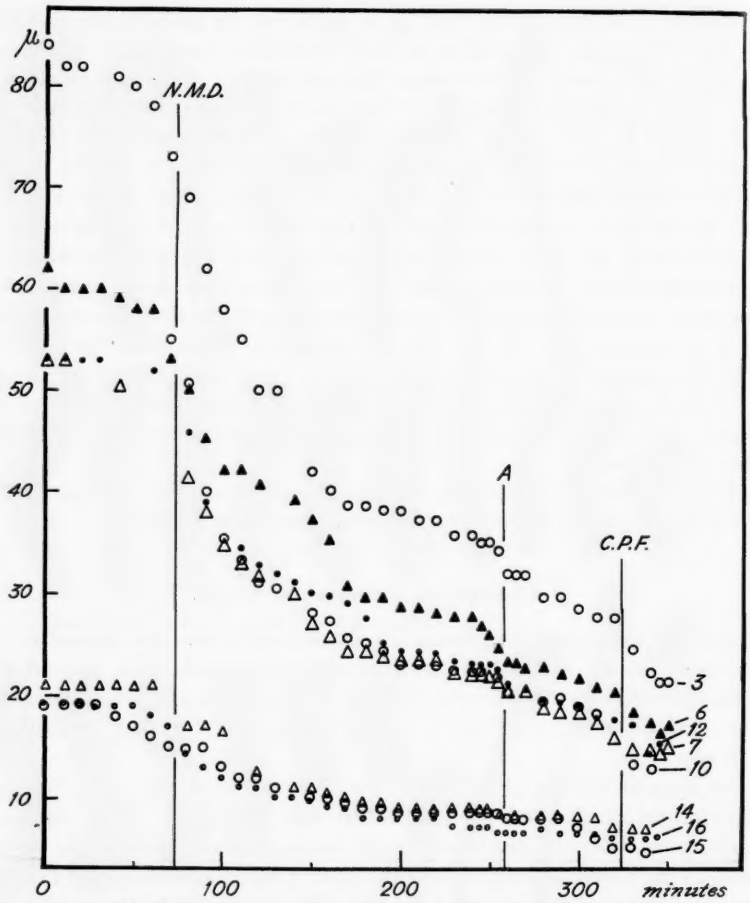

Fig. 5. The change of chromosome length. N. M. D.=dissolution of the nuclear membrane; A=beginning of anaphase; C. P. F.=beginning of the formation of the cell plate. Cell No 405/55; cf. also Figs. 1 a, 2 and 4.

study is contrary to the observations of MANTON (1939) who found in mitosis of *Osmunda* that the chromosome length in anaphase is greater than that in metaphase.

In the endosperm of *Haemanthus katharinae* the chromosomes reach their maximum contraction at the transition stage between anaphase and telophase, a result in full agreement with the observations of BĚLAŘ

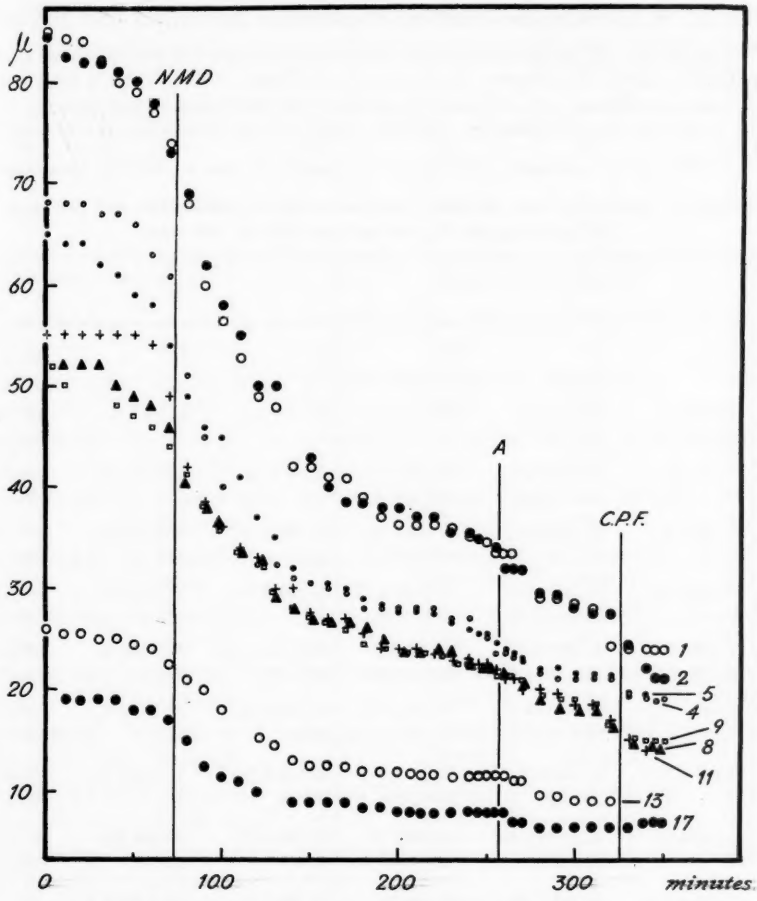

Fig. 6. Continuation of Fig. 5. Some chromosomes from the same cell.

(1929). During telophase *i.e.* simultaneously with the beginning of cell plate formation, there seems to be no further change of chromosome length in some cells, while in others they continue to shorten. However, as a result of structural changes which make the outlines of the chromosomes appear blurred, measurements are not possible in most cases.

The change in length is approximately proportional to the initial length of the chromosomes in prophase. This is clearly shown when the

TABLE 1. *Chromosome length of Haemanthus katharinae (cell 405/55)*

The length in μ of the chromosomes and of their arms (where arm measurements were possible) is given. Homologous chromosomes: the longest — A, 1, 2 and 3, long — B, 4, 5 and 6, medium — C, 7, 8 and 9, medium with subterminal kinetochore — D, 10, 11 and 12. The percentage of change in length calculated according to the fomula: $\frac{l_o - l_x}{l_o} 100$ where l_o=length at 0 time, l_x=length at time x. Nuclear membrane disappears between 70 and 80 mins. Anaphase begins between 255 and 260 mins., cell plate begins to form between 320 and 330 mins.

Time (mins.)	Chromosome type A			Chromosome type B		
	1	2	3	4	5	6
0	85	84 55+29	84 57+27	68 —	65 —	62 —
30	82 (3.5 %) 54+28	82 (2.4 %) 53+29	78 (7.1 %) — —	67 (1.4 %) — —	62 (4.5 %) — —	60 (3.2 %) 41+19
60	77 (9.4 %) — —	78 (7.6 %) 51.5+26.5	75 (10.7 %) 49+26	63 (7.2 %) — —	58 (10.7 %) — —	58 (6.4 %) 39+19
90	60 (29.4 %) 39+21	62 (26.2 %) 41+21	59 (29.7 %) 39+20	45 (33.8 %) — —	46 (29.2 %) 34+12	45 (27.4 %) 35+10
120	49 (42.3 %) 32+17	50 (40.4 %) 34.5+15.5	52.5 (37.5 %) 35+17.5	32 (52.7 %) — —	37 (43 %) 28+9	40.5 (34.6 %) 29+11.5
150	42 (50.5 %) 29+13	42 (50 %) 28+14	43 (49 %) 28.5+14.5	30.5 (55.1 %) 23.5+7	30.5 (53 %) 25+5.5	30.5 (50.8 %) — —
180	39 (54.1 %) 27+12	38.5 (54.1 %) 28+10.5	38 (54.7 %) 25+13	28 (58.6 %) 22+6	29 (55.3 %) 22+7	29.5 (52.4 %) 21+8.5
210	36 (56.4 %) — —	37 (55.9 %) 27+10	36 (57.1 %) 24+12	28 (58.6 %) 22+6	28 (56.9 %) 21+7	28.5 (54 %) 21+7.5
240	35 (58.6 %) 24+11	35.5 (57.7 %) 26+9.5	34 (59.7 %) 22+12	27 (60.3 %) 21+6	26 (60 %) 20+6	27.5 (55.6 %) 20+7.5
270	32 (62.3 %) 22+10	32 (61.7 %) 23.5+8.5	31 (63 %) 22+9	23 (66.1 %) — —	22.5 (65.3 %) 18+4.5	22.5 (63.7 %) — —
300	28.5 (66.4 %) 19+9.5	28.5 (66 %) 19.5+9	27 (67.8 %) 21+6	21 (69.1 %) 17.5+3.5	21.5 (66.9 %) 18+3.5	21.5 (65.3 %) 18+3.5
330	24 (71.7 %) 17.5+6.5	24 (71.4 %) 16+8	19.5 (76.9 %) 16+3.5	19.5 (71.3 %) 15.5+4	19.5 (70 %) 16+3.5	18 (70.9 %) 15+3
345	24 (71.7 %) — —	21 (75 %) 14+7	18 (78.5 %) — —	19 (72 %) — —	19 (70.7 %) 15+4	16 (74.1 %) 13.5+2.5
350	24 (71.7 %) — —	21 (75 %) — —	18 (78.5 %) — —	19 (72 %) — —	19 (70.7 %) — —	17 (72.5 %) — —

Table 1 (*continued*)

Time (mins.)	Chromosome type C			Chromosome type D		
	7	8	9	10	11	12
0	53	53	52	52	55	53
	—	—	—	49+3	—	—
30	—	52 (1.8%)	—	50 (4 %)	55 (0 %)	53 (0 %)
	—	— —	—	48+2	52+3	— —
60	—	48 (9.4 %)	46 (11.5 %)	48 (7.6 %)	54 (1.8 %)	52 (1.8 %)
	—	31+17	— —	46+2	51+3	— —
90	38 (28.3 %)	38 (28.3 %)	38 (26.9 %)	40 (23 %)	38.5 (30 %)	39 (26.4 %)
	— —	25+13	— —	38+2	— —	37+2
120	31.5 (40.5 %)	33 (37.7 %)	32.5 (37.5 %)	31 (40.3 %)	33 (40 %)	33 (37.7 %)
	— —	24+9	— —	29+2	31+2	— —
150	27 (49 %)	27 (49 %)	27 (48 %)	28 (41.6 %)	27.5 (50 %)	30 (43.4 %)
	— —	20.5+6.5	— —	26+2	26+1.5	27+3
180	24 (54 %)	26 (50.9 %)	24.5 (52.8 %)	25 (51.9 %)	25.5 (53.5 %)	27.5 (48.1 %)
	— —	19.5+5.5	— —	22.5+2.5	23.5+2	25+2.5
210	23 (56.6 %)	24 (54.6 %)	24.5 (52.8 %)	23 (55.7 %)	24 (56.3 %)	24.5 (53.7 %)
	— —	18+6	— —	20.5+2.5	22.5+1.5	22+2.5
240	22 (58.4 %)	23 (56.6 %)	23 (55.7 %)	22 (57.6 %)	23 (58.1 %)	23 (56.6 %)
	— —	17.5+5.5	— —	20.5+1.5	22+1	21+2
270	20 (62.2 %)	20.5 (61.3 %)	21 (59.6 %)	21 (56.9 %)	20 (63.6 %)	20.5 (59.6 %)
	— —	15.5+5.5	— —	19.5+1.5	19+1	19+1.5
300	18 (66 %)	18 (66 %)	19 (63.4 %)	19 (63.4 %)	18.5 (66.3 %)	19 (64.1 %)
	— —	13.5+4.5	— —	18+1	17+1.5	18+1
330	14.5 (72.6 %)	15 (73.5 %)	15 (71.1 %)	13 (77.9 %)	15 (72.7 %)	17 (67.9 %)
	— —	13+2	— —	12+1	13.5 +1.5	15.5+1.5
345	14 (73.5 %)	14 (73.5 %)	15 (71.1 %)	—	—	15 (71.6 %)
	— —	12+2	— —	—	—	— —
350	15 (75.4 %)	—	15 (71.1 %)	—	—	14 (73.5 %)
	— —	—	— —	—	—	— —

change in length is expressed as percentage — Table I, and especially when logarithmic values of chromosome length are plotted (Figs. 9 and 10). This result seems to be quite different from that found by SVÄRDSON (1945) and WICKBOM (1949) — *cf.* discussion. In the endosperm the chromosomes of different length behave in the same general way. However, there are differences in the finer details of the course of contraction, even between homologous chromosomes in the same cell;

Table 1 (continued)

Time (mins.)	Small chromosomes					
	13	15	16	17	18	20
0	26 18+8	19 10.5+8.5	19 —	19 —	18 —	21 —
30	25 (3.8 %) 17+8	19 (0 %) — —	19 (0 %) — —	19 (0 %) 15+4	17 (5.8 %) — —	21 (0 %) — —
60	24 (7.6 %) 17+7	16 (16.7 %) — —	18 (5.2 %) 12+6	18 (5.2 %) 14.5+3.5	16 (11.7 %) — —	21 (0 %) 14+7
90	20 (23 %) 13.5+6.5	15 (21 %) 9+6	13 (31.5 %) — —	12.5 (34.2 %) — —	13 (27.7 %) — —	16.5 (21.4 %) 11.5+5
120	15 (42.3 %) — —	12 (36.8 %) 6.5+3.5	11 (42.1 %) 7.5+5.5	10 (47.3 %) — —	— — — —	12 (42.8 %) — —
150	12.5 (51.9 %) — —	10 (47.3 %) 5.5+4.5	10 (47.3 %) 6.5+3.5	9 (52.6 %) 6+3	9.5 (47.2 %) — —	11.5 (42.5 %) 9+2.5
180	12 (53.8 %) — —	9 (52.6 %) 5+4	8 (57.8 %) 6+2	8.5 (55.2 %) 6+2.5	8.5 (52.7 %) — —	9.5 (54.7 %) 7+2.5
210	11.5 (55.7 %) — —	8.5 (55.2 %) 5+3.5	8 (57.8 %) 5+3	8 (57.8 %) 6+2	8 (55.5 %) — —	9 (57.1 %) 7+2
240	11.5 (55.7 %) 7.5+4	8.5 (55.2 %) 5+3.5	7 (63.1 %) 4.5+2.5	8 (57.8 %) 6+2	7 (61.1 %) — —	9 (57.1 %) 6.5+2.5
270	11 (57.6 %) 7+4	8 (57.8 %) 4.5+3.5	6.5 (65.7 %) 4.5+2	7 (63.1 %) 5.5+1.5	6.5 (63.8 %) — —	9 (57.1 %) 6.5+2.5
300	9 (65.3 %) 6.5+2.5	7 (63.1 %) 4.5+2.5	6.5 (65.7 %) 4.5+2	6.5 (65.7 %) 5+1.5	6 (66.6 %) — —	9 (57.1 %) 6+3
330	— — — —	5 (73.6 %) 4+1	6 (68.4 %) 4+2	6.5 (65.7 %) 5+1.5	5 (72.2 %) — —	8 (61.9 %) 6+2
345	— —	— —	— —	7 (63.1 %) 5+2	— — — —	9 (57.1 %) — —
350	— —	— —	— —	7 (63.1 %) — —	— — — —	9 (57.1 %) — —

their dimensions at the same stages may not be exactly the same. It should therefore be stressed that though the general course of the changes is the same, different chromosomes may shorten slightly differently.

During c-mitosis caused by the influence of colchicine the chromosomes are more strongly contracted already in prophase than they are during normal mitosis. Furthermore, the duration of c-mitosis is much

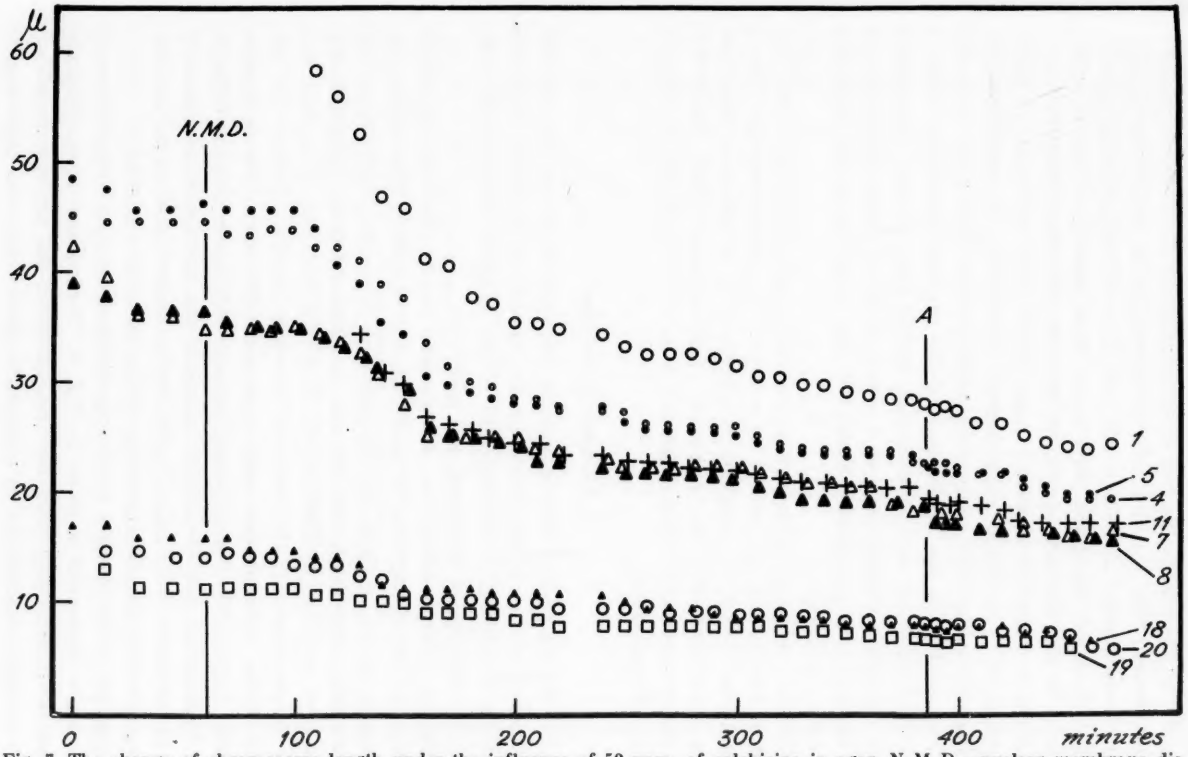

Fig. 7. The change of chromosome length under the influence of 50 ppm. of colchicine in agar. N. M. D.=nuclear membrane disappearance. *A*=c-anaphase, *i. e.* the sudden lapse of chromatid pairing and formation of "ski pairs". The micrographs of this cell are in the work of MOLÈ-BAJER (1958), cell No 207/56.

longer. For this reason the rate of the changes in chromosome length is much slower (Fig. 7). Also, as the contraction of chromosomes in c-mitosis was studied in a single cell only, it is difficult to judge whether or not, there are consistent differences from normal mitosis in certain features of the contraction pattern. In early metaphase there is during c-mitosis a period of relatively rapid contraction that most probably corresponds to the rapid prometaphase contraction of normal mitosis. In the cell studied this contraction period occurs later than in normal mitosis relative to the breakdown of the nuclear membrane. After a period of relatively slow contraction during most of the c-metaphase there are distinct indications of a short period of rapid contraction at the beginning of c-anaphase, similar to that occurring at the beginning of normal anaphase.

The contraction pattern during c-mitosis is not clearly different from that of normal mitosis, except in so far as the occurrence of a strong contraction already in prophase is concerned. A difference of this kind is in agreement with the suggestion of ÖSTERGREN (1944, 1951) that colchicine may have a direct influence on the contraction mechanism of the chromosomes.

3. Changes in volume

After the length had been measured, attempts were made to calculate the volume of the chromosomes; *i.e.* the volume of the single chromatid until the beginning of anaphase and of the daugther chromosome (two half-chromatids) in anaphase and telophase.

The two chromatids of the chromosome can be distinctly seen from prophase until metaphase. In anaphase and especially in telophase the structure is not so clear, *cf.* discussion p. 594. The thickness of two chromatids seen lying side by side at prophase and metaphase was measured and then divided by two to get the thickness of a single one. The measurements of the thickness varied in accuracy because the outlines of the chromosomes were seen more distinctly in later than in earlier stages. Two volumes were always calculated, corresponding to the highest and lowest possible values of the measured thickness *e.g.* for the diameter 1.6 μ and 1.7 μ the two extreme values of the chromosome diameter in prophase being in this case 3.2 μ and 3.4 μ.

A graph of the changes in chromatid thickness and in the volume of the single chromatid is given in Fig. 8. The volume of a chromatid increases immediately after the dissolution of the nuclear membrane and reaches a maximum in metaphase, just before the beginning of

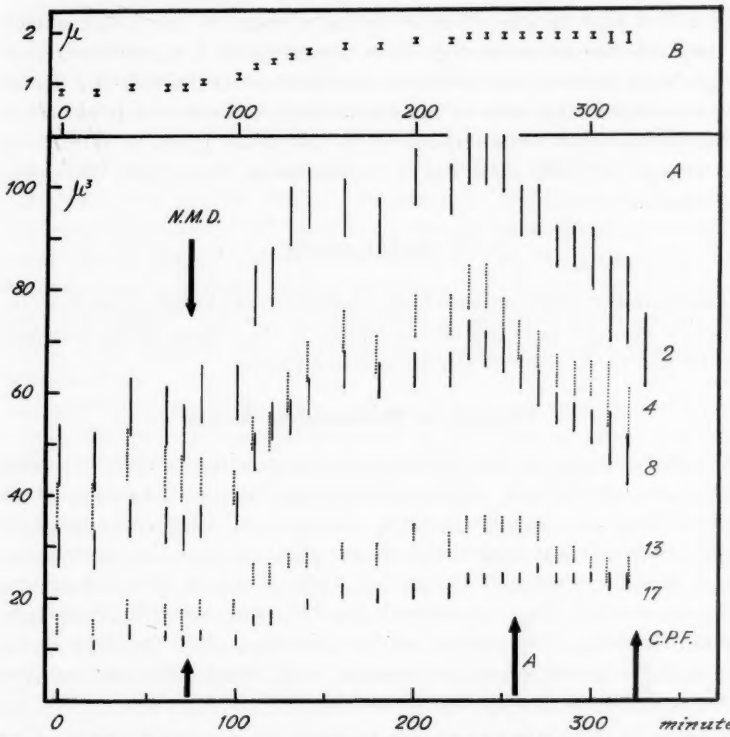

Fig. 8. *A.* The change of volume of the chromatid (until beginning of anaphase) and of the chromosome during anaphase. The two ends of each line represent the two volumes calculated for the two possible extreme values in the estimation of the thickness of the chromosome.

B. The change in the thickness of the chromatid. The thickness of the whole chromosome (until the beginning of anaphase) was measured and divided by two to obtain the value for the chromatid.

anaphase, when the chromosomes begin to decrease in length again. The volume begins to decrease simultaneously with the start of chromosome contraction just before anaphase and it decreases during the whole of anaphase. The volume of the chromatid of chromosome no. 2 (Figs. 1, 2 and 8) is about 50 μ^3 in prophase, about 105 μ^3 in metaphase just before the beginning of anaphase, and about 70 μ^3 at the start of the cell plate formation.

The changes in late anaphase and telophase are more complicated. The thickness of the chromatids is much less uniform than in other

stages. This may be a result of structural changes in the chromosomes: in anaphase the chromosomes, each consisting of a single chromatid, are probably divided into two half-chromatids, and the half-chromatids may separate at the ends of the chromosomes. Thus, the thickness of some chromosome arms, especially at the distal parts, is larger, *e.g.* Fig. 2 time 320′, 340′, and Fig. 3, chromosome No. 6, time 300′—340′ and others.

IV. DISCUSSION

There are three factors which may influence the length of the chromosomes: 1. changes in chromonema length, 2. the course of the spiralization cycle and, 3. changes of chromosome volume.

1. Changes in chromonema length

Detailed studies on the changes of chromonema length in meiosis were done by BELLING (1928), MANTON (1939) and SPARROW *et al.* (1943). They all conclude that the chromonema length diminishes in certain stages. It was suggested, however, that in mitosis the chromonema length does not change (SAX and SAX 1935, MANTON 1939). At present, however, MANTON does not support this statement (according to personal communication). The present author considers that the data in the literature do not permit any conclusion as to whether the chromonema changes in length during mitosis or not.

2. The spiralization cycle

This factor is partly responsible for changes in length of the meiotic chromosomes. The increase in diameter of the chromonema spiral and the decrease in number of its gyres causes a shortening of the chromosomes at meiosis in *Osmunda* (MANTON 1949). This factor may also play an important role in mitosis. The diameter of the chromatids increases in mitosis, but so far no data concerning the number of chromonema gyres are available. In the endosperm of *Haemanthus* the lengths of the individual telophase chromosomes change approximately in the same way, but even homologous chromosomes in the same cell are often of slightly different dimensions. This is also indicated by the measurements of POWERS (1942) who made very precise measurements of chromosome length (error 0.08 μ).

However, in the opinion of the present author the differences are by far too small to indicate that the coiling in the different parts of the

chromosomes in *Haemanthus* is not the same. Differences larger than 10 % occurred in 5 chromosomes, only, out of 24 measured (Table 1) and they never surpassed 16 %. It should be stressed that these differences of 10 % and more were found in homologous chromosomes. In the author's opinion these differences are too large to be considered as due to the error of measurement, which though difficult to estimate, probably is not greater than a few percent. The measurements were made on very flattened cells and, even though the mechanical conditions permit the general course of mitosis to be normal, the shortening of the chromosomes may meet with some difficulties. The mechanical condition in flattened cells are not uniform in different parts of the cell, which may explain the differences in length between homologous chromosomes.

It is possible that there may exist some types of changes in chromosome length which are characteristic of different species. Thus, the pattern of the change of chromosome length in prophase of *Leucojum* differs considerably from that of *Haemanthus* (Fig. 9). MANTON (1939) found that the length of anaphase chromosomes of *Osmunda* is larger than that of the metaphase ones, while in the endosperm of *Haemanthus* they decrease in length continously throughout mitosis except for a short interval in metaphase. LONGLEY (1941) found that the coiling system of some chromosomes in a set may be different, thus, *e.g.* the B-chromosomes in maize show distinctly a premature shortening. The opinion that the pattern of coiling may be different in different materials is also suggested by a comparison of the measurements on *Haemanthus* and *Leucojum* with those on animal material made by SVÄRDSON (1945) and WICKBOM (1949). SVÄRDSON made very exact measurements on fixed chromosomes of *Salmo alpinus* between late prometaphase and the beginning of anaphase. He found that the chromosomes shorten between these stages and that chromosome arms of different size shorten at different rates, the long arms of the chromosomes showing a greater relative contraction than the short ones. He suggests that: "the contraction in both arms proceeds independently of one another, *i. e.* the contraction cannot pass the centromere. Thus if the two arms of the chromosome are of unequal length, the index showing the relative length of the arms will be modified" (p. 25). He explains this on the assumption that the shorter chromosomes iniatially contract more rapidly than the longer ones. SVÄRDSON was unable to measure the chromosomes of the earlier stages but this was done by WICKBOM on *Bufo bufo* (early prophase to metaphase), *Salamandra salamandra* and *Hyla arborea.*

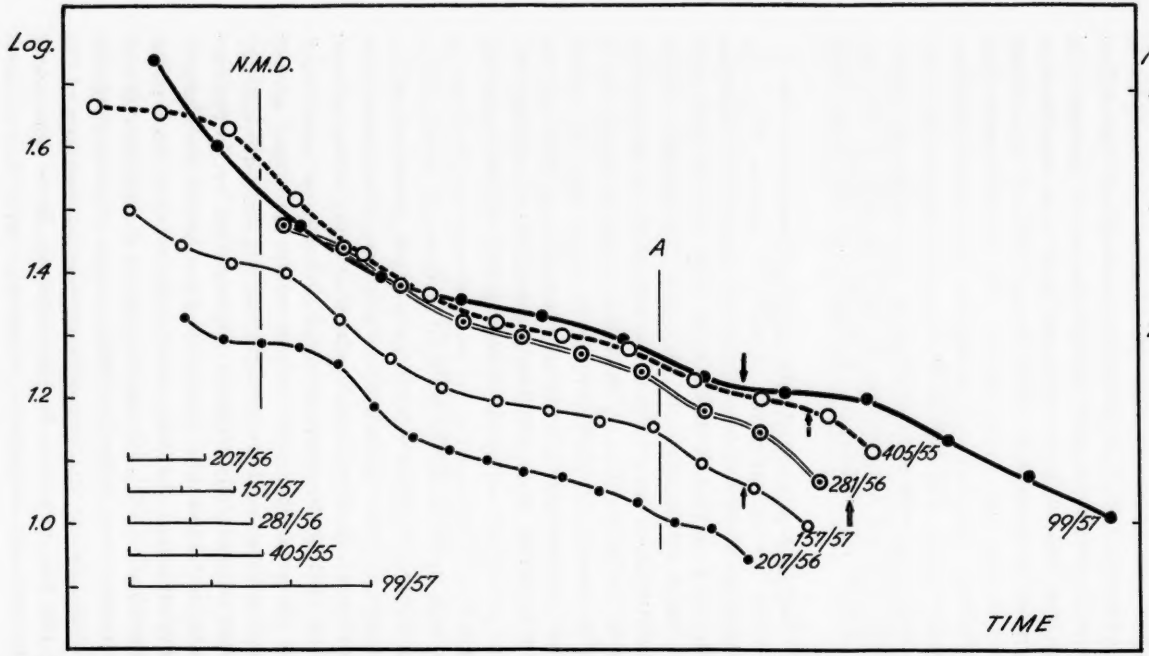

Fig. 9. Change of chromosome length in different cells. Mean values of chromosome length (logarithmic values) of all chromosomes measured from each cell plotted against the time. Time scale different for different cells, to make them comparable *i. e.* the distance between N. M. D (dissolution of the nuclear membrane) and A (beginning of anaphase) is the same time; scales given below, each scale corresponding to 1 hr. The arrows mark the beginning of cell plate formation. *Haemanthus katharinae* cells Nos. 157, 281 and 405 (normal) and 207 (colchicine treated); *Leucojum aestivum* — No 99. The curve for cell No 207 is displaced 0.1 log unit below the scale. The pattern of change of length in all *Haemanthus* cells including the colchine treated cell, is very similar. The change in prophase in *Leucojum* shows some differences *i. e.* there is no change in the rate of decreasing in length at the disappearance of the nuclear membrane.

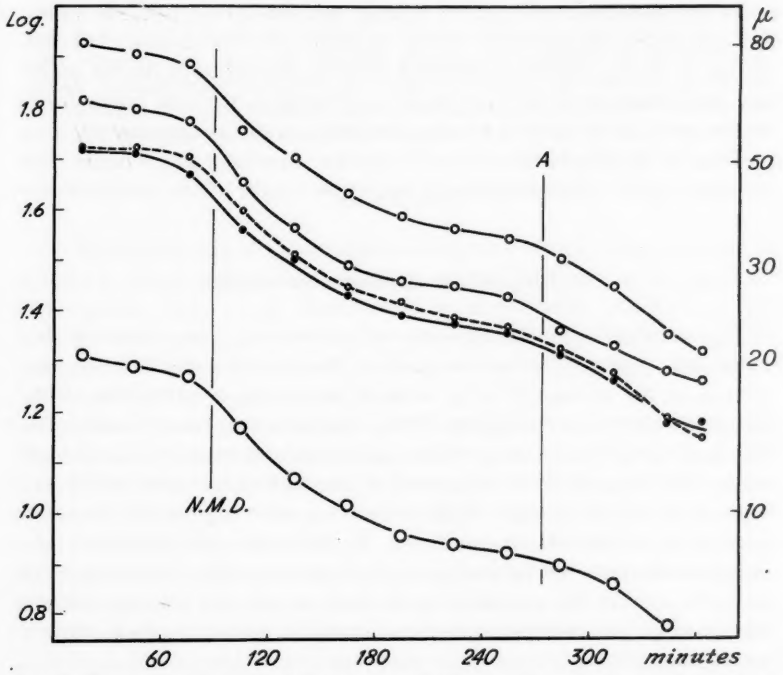

Fig. 10. Chromosome length plotted on a logarithmic scale against the time. Each of the four upper curves represents mean values of three homologous chromosomes (Fig. 5—6) *i. e.* groups A, B, C, and D (Table I). The lowest curve, mean values of the chromosomes Nos: 13, 15, 16, 17, 18 and 20. N. M. D. — nuclear membrane disappearance. A — beginning of anaphase.

He found that "between early prophase and metaphase all chromosomes are shortened by the same percentage irrespective of length. Between mid-prophase and metaphase the shortening is proportionally much greater in long chromosomes than in small ones. Thus between early prophase and mid-prophase the opposite must have taken place. The shortening must have been proportionally stronger in small chromosomes than in long ones" (p. 247—248). This supports SVÄRDSON's findings and hypothesis. In *Haemanthus* the measurements performed on living chromosomes between late prophase and telophase indicate, on the contrary, that all the chromosomes shorten in a similar way in all the stages independently of their dimensions. This is especially clearly shown when the logarithmic values of the length of long and

short chromosomes are plotted against the time (Fig. 10). The curves are approximately parallel, which supports this conclusion. The data (Table 1) on the length of different arms in *Haemanthus* do not permit any conclusions as to whether there is a change of the arm ratios during the progression of mitosis, because the error in the measurements of the position of the kinetochore is much greater, especially in prophase, than the error in the measurements of the whole length of the chromosomes.

3. Changes in chromosome volume

The calculation of chromosome volume contains an error which is difficult to estimate. It was assumed in the present study that the cross section of the chromatid is a circle as was done by BELLING (1928), MANTON (1935) and SVÄRDSON (1945). In the endosperm of *Haemanthus* this may not be true during late anaphase and telophase as the chromosomes then appear to be composed of two half-chromatids which may separate from one another at the ends. This error is probably especially important during the later stages. Furthermore, the diameter of a chromosome may not be the same all along its length. Therefore in the author's opinion the graphs given here show only the general course of the changes in chromosome volume and the numerical data are only approximate.

The changes of volume may be caused by changes in the chemical composition of the chromosomes. There is a rapid drop of chromosome mass before the disappearance of the nuclear membrane and during prometaphase until metaphase (RICHARDS and BAJER, 1960). There is also a continuous incrase of chromosome concentration (per unit area of a chromosome) from prometaphase until late anaphase, when it shows a tendency to decrease (AMBROSE and BAJER, 1960). A change of RNA content during anaphase was shown to occur in the chromosomes of animal cells by JACOBSON and WEBB (1952). SHIMAMURA and OTA (1956) demonstrated this change also in plant cells. In *Haemanthus* the changes in volume may be caused either by the release of some substances from the chromosomes during anaphase, or by the swelling or shrinkage of certain parts of the chromosomes, *i.e.* by changes of water content. The processes of hydration and dehydration seem to play an important role in mitosis, as was stressed especially by Japanese workers. A review on the subject was given by KUWADA (1939).

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SUMMARY

(1) Measurements of the changes in length of the chromosomes during mitosis in living cells and calculations of their volumes, from prophase to telophase, were made. Endosperm of *Haemanthus katharinae* and *Leucojum aestivum* was used as the material. The measurements of the length were made on four cells undergoing normal mitosis and on one cell under the influence of colchicine.

(2) The chromosomes begin to decrease rapidly in length before the dissolution of the nuclear membrane and continue to decrease in length until metaphase. In metaphase there may be a short period when the chromosomes do not change their length. They start to decrease in length again before the start of anaphase and continue to decrease throughout the whole of anaphase. It is possible that under the influence of colchicine the contraction pattern is slightly modified.

(3) Homologous chromosomes may have slightly different lengths in different cells (and even in the same cell) at corresponding stages and also the course of changes may not be exactly the same.

(4) The volume of the chromosomes increases until late metaphase when it reaches a maximum, begins to decrease before the start of anaphase and continues to decrease during the whole of anaphase.

(5) Some factors which may be responsible for the changes of volume and length of the chromosomes are discussed.

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SWEET TASTE PERCEPTION FOR PHENYLTHIOUREA (P. T. C.)

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INTRODUCTION

THE observation by FOX (1931) that phenylthiourea (phenylthiocarbamide) was tasteless to some persons while most people tasted the compound as bitter was followed by a genetic analysis by BLAKESLEE and SALMON (1931). Their findings suggested an autosomal recessive mode of inheritance for taste blindness to P. T. C. BLAKESLEE (1932) observed that some persons described the taste of P. T. C. by some term other than bitter, including sour and sweet. RICHTER and CLISBY (1941) reported that among 261 persons 15 (5.75 %) described subliminal solutions of P. T. C. as sweet. Likewise HARRIS and KALMUS (1949) remarked that some persons characterized P. T. C. as sweet, sour, salty, for example; this particularly so near the taste thresholds of these persons. Examining Japaneses with P. T. C. powder CHIKAUCHI (1952) noted that a few persons found the taste sweet or salty. LUGG (1955) found 3 Malayan Negritos to describe weak solutions of P. T. C. as sour; one of these persons tasted solution 14 (HARRIS' and KALMUS' denomination, 1949) as sweet. In their research series LUGG and WHYTE (1955) noted, among other exceptional taste qualities for P. T. C., sweet taste for this substance in 0 to 2 per cent of the Europeans and Asians. Of 50 Senois in Malaya 8 per cent tasted P. T. C. as sweet at or close to their taste threshold (LUGG 1957).

DAS (1958) reported from Calcutta that non-bitter tasters for P. T. C. might not exceed 1 to 2 per cent of the population. He suggested that it might be useful to record such abnormal tasters in various populations and to take up the question of inheritance of such variations by means of family studies. In agreement with this view the frequency of sweet tasters for P. T. C. has been investigated among adolescents in South Sweden, and family data on such sweet tasters have been collected.

Material

The material consisted of grammar or high-school pupils in Malmö, Sweden, of these, 816 were boys aged 14 to 21, mean age 16.7 ± 1.5 (S.D.) years and 666 girls, aged 14 to 20, mean age 16.2 ± 1.2 years. The overall mean age of the pupils was 16.5 ± 1.4 years. The parents and sibs of sweet tasters were visited at home and, when possible, examined, 74 % cooperated.

Method

P. T. C. solutions were prepared in accordance with HARRIS and KALMUS (1949) but with distilled water instead of boiled tap water. Their method implies that from a stock solution, containing 1300 mg. P. T. C. per litre solution, additional solutions are prepared by diluting by, $^1/_2$, $^1/_4$, $^1/_8$ etc. The solutions were numbered 1—13, 1 for the stock solution and 13 for the most dilute solution. I used separate beakers made of paraffined paper for each solution. All solutions were kept at room temperature.

In the first stage of the study each pupil was tested with solution 10, non-tasters or non-bitter tasters for this solution were tested with solution 7. Any non-tasters or non-bitter tasters of solution 7 were then tested with solution 1. Examination with a wider variety of solutions would have interfered too much with their lessons.

The second stage of the investigation comprised the sweet tasters secured in this way as well as their parents and sibs. They were tested with a complete series of P. T. C. solutions and with the battery of 7 compounds listed in Table 1.

TABLE 1. *Solutions used for examination of sweet tasters and members of their families.*

Compound	Solution a	Solution b	Solution c
Dulcine	0.00085 %	0.00170 %	0.01700 %
Sucrose	0.1 %	0.2 %	2.0 %
Quinine sulphate	0.00005 %	0.00010 %	0.00100 %
Strychnine	0.0000004 %	0.0000008 %	0.0000080 %
Allylthiourea	0.000586 %	0.001172 %	0.011720 %
Hydrocloric acid	0.0015 N	0.0030 N	0.0300 N
Sodium chloride	0.04 %	0.08 %	0.80 %

As indicated in the table, solutions b were twice as concentrated as solutions a, and solutions c were ten times the concentration of b. These

TABLE 2. *Taste quality of P. T. C. as described by 1482 school pupils.*

Age	14		15		16		17		18		19		20		21		Total		Total	Percentage		
Sex / Taste quality	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	Both sexes	M	F	Both sexes
Bitter	9	17	164	149	225	194	141	130	111	60	76	23	24	5	5		755	578	1333	92.52	86.79	89.95
Tasteless						2			1		1						2	2	4	0.24	0.30	0.27
Sweet	2		8	5	11	8	3	8	3	3	2				1		30	24	54	3.68	3.60	3.64
Sour			2	8	3	13	2	6	2	1	2						11	28	39	1.35	4.20	2.63
Salt		1	3	13	3	14	7	4	4	1		1	1				18	34	52	2.21	5.11	3.51
Total	11	18	177	175	242	231	153	148	121	65	81	24	25	5	6		816	666	1482			
Total	29		352		473		301		186		105		30		6		1482					

solutions were used in the same way as when testing the pupils with P. T. C in the first stage of the study. Thus non-tasters or inadequate tasters of a weak solution were re-tested with a more concentrated solution. Tasting for a given substance was said to be inadequate if it deviated from that described by the majority of the control persons recorded in Table 3.

In the family examinations it was rarely possible to test individual family members in privacy; they were told that different persons conceive the taste of the solutions differently, and the youngest members of the family were examined first.

RESULTS

The first stage of the investigation revealed 54 of the 1482 (3.64 %) school pupils to be sweet tasters. Sweet tasting did not vary with sex (Table 2). In Table 2 the rows Sweet, Sour and Salt represent pupils who tasted any of the three P. T. C. solutions as indicated. These taste qualities were mutually exclusive. However, bitter taste was perceived

TABLE 3. *Taste quality of test compounds as described by 22 control persons.*

Compound and concentration	Male	Female	Both sexes	Taste sensation not adequate to compound
Dulcine a	7	10	17	Sour (1), indefinite (2)
b	3	1	4	
c	1	0	1	
Sucrose a	4	5	9	Bitter (2), indefinite (1)
b	4	4	8	Bitter (4), indefinite (4)
c	3	2	5	
Quinine sulphate a	7	7	14	Sweet (2), sour (1), indefinite (1)
b	2	0	2	Sweet (1), indefinite (1)
c	2	4	6	Sweet (1)
Strychnine a	9	9	18	Sweet (7)
b	1	2	3	Sweet (2)
c	1	0	1	
Allylthiourea a	9	9	18	Sweet (4), sour (1), indefinite (5)
b	0	1	1	Sweet (3), sour (1), salt (1, indefinite (1)
c	2	1	3	Sweet (1), sour (1)
Hydrochloric acid a	8	10	18	Bitter (2), salt (1), indefinite (4)
b	3	1	4	Bitter (1), indefinite (2)
c	0	0	0	
Sodium chloride a	4	4	8	Indefinite (2)
b	4	5	9	Indefinite (3)
c	3	2	5	

by all other tasters when they were tested with the most concentrated solution. Thus, the corresponding row represents all tasters that described P. T. C. exclusively as bitter. The remaining 4 persons reported no taste sensation for any of the P. T. C. solutions.

Sour tasting and salt tasting for P. T. C. was more common among the females, with $\chi^2=11.674$ and P less than 0.001 in the case of the sour tasters and $\chi^2=9.104$, P between 0.01 and 0.001 for salt tasters (1 D. F.)

Table 3 shows the results of testing control persons with the solutions listed in Table 1. This control series consisted of 11 men, aged 22 to 49, and 11 women, aged 17 to 78.

All control persons could taste solutions c adequately at these concentrations except three men. One of them described all solutions of quinine sulfate as sweet. He was also a sweet taster for P. T. C., the only one of the control series. The second tasted a solution c of allythiourea as sweet, and the third, as sour. The three middle columns in Table 3 give the number of control persons who assessed the solutions of the respective rows as the weakest concentrations they could taste under the experimental conditions.

TABLE 4. *Sibs of 37 sweet tasters by taste quality for P. T. C.*

	Taste quality		
Sex	Sweet	Non-sweet	Total
Male	13	10	23
Female	11	10	21
Both sexes	24	20	44

Table 4 gives Data on sibs of all the 37 pupils who were sweet tasters. Comparison between Tables 4 and 6 shows agreement between the observed frequency of 54.55 % sweet tasters among sibs of probands and the frequency among such family members at the assumed prevalence of 7.16 % sweet tasters in the general population (see Discussion).

TABLE 5. *Sibs of 26 sweet tasters with at least one parent sweet taster for P. T. C.*

	Taste quality		
Sex	Sweet	Non-sweet	Total
Male	9	7	16
Female	7	5	12
Both sexes	16	12	28

In Table 5 offspring of 2 matings between non-sweet tasters are excluded, numbers B I and B III in Appendixes A and B. It should be observed that the father of family B I described an indefinite taste that was not bitter for P. T. C. solutions 4 to 6, and that both parents of family B III stated such undecided taste sensations, each for two different P. T. C. solutions. Further, the mother of family B I was one of the two persons found not to have any taste sensation when tested with the most concentrated quinine sulfate solution; she was also a non-taster for all P. T. C. solutions. I also excluded 4 incomplete families (A II, D VI, E IV, E V) and 5 families (B VI, C IV, C VII, C VIII, and E VII) of which one or both parents were sweet tasters for quinine sulfate and, in addition, tasted at least one P. T. C. solution as indefinite. After these exclusions 57.14 % sweet tasters occurred among the sibs of the probands, in agreement with the expected value (Table 6) on the assumption of 7.16 % sweet tasters in the general population.

TABLE 6. *Frequency of carriers of a dominant trait calculated according to* HULTKRANTZ *and* DAHLBERG *(1927).*

Frequency of dominant trait carriers		
In the population	Among sibs	Among parents
$1-q^2$	$1-\frac{q^2(3+q)}{4(1+q)}$	$1-\frac{q^2}{1+q}$
3.64	51.60	51.37
7.16	53.15	52.72

Table 6 presents the frequency of carriers of a dominant trait, as calculated according to HULTKRANTZ and DAHLBERG (1927). The frequency value 3.64 % is the incidence of sweet tasters among school pupils observed in the present study. The value 7.16 % is assumed on grounds given under Discussion.

TABLE 7. *Parents of 33 sweet tasters for P. T. C. from complete families.*

	Taste quality		
Sex	Sweet	Non-sweet	Total
Male	13	20	33
Female	17	16	33
Both sexes	30	36	66

Table 7 includes all complete families. The same families are excluded from Table 8 as from Table 5. Agreement was found between expected

and observed number of sweet tasters (Tables 6, 7 and 8). Neither among parents nor among sibs did the frequency of sweet tasters for P. T. C. vary with sex.

TABLE 8. *Parents of 26 sweet tasters from complete families with at least one parent sweet taster for P. T. C.*

	Taste quality		
Sex	Sweet	Non-sweet	Total
Male	13	13	26
Female	17	9	26
Both sexes	30	22	52

TABLE 9. *Families of 33 sweet tasters for P. T. C., by progeny and mating type.*

		Sibs of sweet tasters		
Mating type	Number	Sweet taster	Non-sweet taster	Total
Non-sweet×non-sweet	7	5	4	9
Non-sweet×sweet	22	14	11	25
Sweet×sweet	4	2	1	3
All mating types	33	21	16	37

In Table 9 the non-sweet tasters include 7 parents from 5 different matings who were sweet tasters for quinine sulfate and tasted at least one P. T. C. solution as indefinite. Those 5 matings are not included in Table 10.

TABLE 10. *Families of 28 sweet tasters for P. T. C., by progeny and mating type.*

		Sibs of sweet tasters		
Mating type	Number	Sweet taster	Non-sweet taster	Total
Non-sweet×non-sweet	2	2	2	4
Non-sweet×sweet	22	14	11	25
Sweet×sweet	4	2	1	3
All mating types	28	18	14	32

In Figure 1 all sweet tasters for P. T. C. comprised in the family investigation are represented by uninterrupted lines over the solutions (notation according to HARRIS and KALMUS, 1949) they tasted as sweet, and by dotted lines over the solutions they characterized as "perhaps sweet" or indefinite. The vertical dotted lines mark the test solutions that were used at the first stage of the investigation.

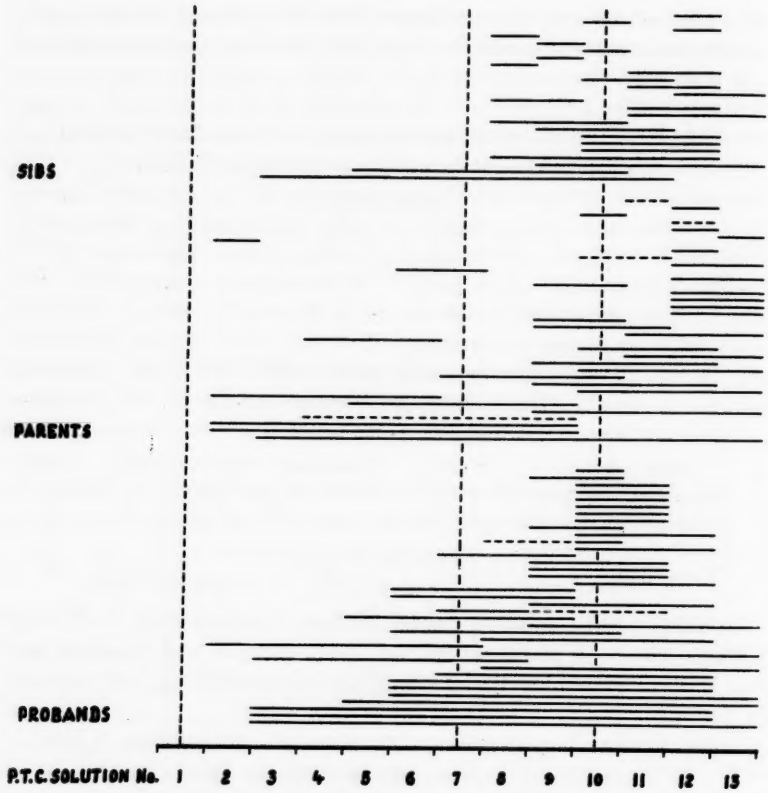

Fig. 1. Range of sweet taste perception for P. T. S. Solutions according to HARRIS and KALMUS (1949).

DISCUSSION

Of 1482 school pupils, 145 (9.78%) described other taste qualities for phenylthiourea than bitter. Of these deviant tasters 54 (37.24 %) were sweet tasters, representing 3.64 % of the whole series of pupils. Here sweet taster is to be understood as a person who described any solution of P. T. C. as sweet, before, after or simultaneously with a bitter taste, and who distinguished sweet from bitter when tested with various compounds. Of the above mentioned 54 sweet tasters one or both of the parents of 26 of the 34 complete families who cooperated

were also sweet tasters. When parents and sibs were included at least one other family member was a definite sweet taster in all instances but 3.

When the investigation was planned it was suspected that inaccurate denominations for taste qualities might occur. HARTMANN (1939) observed that stupid and primitive persons were prone to characterize the taste of P. T. C. by a quality other than bitter. It was partly for this reason that I selected higher schools for the present investigation. However, even then some called sour bitter. Confusion between sour, bitter and even salty tastes by untrained persons, as noted by BÖRNSTEIN (1940) was eliminated as a disturbing factor in the present investigation by the choice of sweet taste for study. In contrast with the three other taste qualities, it was not apt to be called "strong", "bad" or, synonymous with these expressions, "bitter". However, none of the subjects partaking in the family investigation were classified as sweet tasters solely on the ground that they called a P. T. C. solution sweet. They had proved that they could distinguish between sweet and bitter when tested with the compounds listed in Table 1. In addition, all participants of the family study were tested with the whole set of P. T. C. solutions and all but one tasted one of the more concentrated solutions as bitter. That was a parent who was, however, a bitter taster for strychnine. Thus no importance was attached to misnaming of the taste sensation in the classification of the subjects as sweet or non-sweet tasters.

The method applied to the first stage of this study did not exclude false statements as to the taste perception for the three P. T. C. solutions used when testing school pupils. All conscious suggestion was carefully avoided by the examiner, pupils were told to make sure of their true taste perception not only when they described tastes other than bitter, but sometimes also when they promptly reported a bitter taste. This was to avoid the notion being spread that the examiner was only satisfied when they reported a bitter taste. However, the majority of the pupils were bitter tasters, and those not yet tested got to know that P. T. C. "should" taste bitter. This seems to explain the observation of only 168 non-tasters for solutions 7 and 10, *i. e.* 11.34 %, whereas about 30 % should be expected if HARRIS' and KALMUS' (1949) observations, which was actually observed by ÅKESSON (1959) to be approximately correct held good for the population under study. There is thus reason to suspect that a few sweet tasters as well as some non-tasters among the school pupils falsely reported a bitter taste. If so, the observed frequency of 3.64 % sweet tasters would be erroneously low.

Compared with the sparse data available in the literature, the observed

rate of sweet tasters would seem rather high, surpassed only by RICHTER's and CLISBY's (1941) observation of 5.75 % sweet tasters and by LUGG's (1957) observation of 8 % sweet tasters among 50 Senois.

Figure 1 shows that sweet tasters perceived a wide variety of P. T. C. solutions as sweet. With the technique of the first phase of the present study 12 of the sibs and 17 of the parents of sweet tasters would have been missed, *i. e.* 49.15 % of 59 sweet tasters among the family members of the probands. In the absence of a more accurate estimate of the frequency of sweet tasters in the general population, the observed frequency among school pupils, 3.64 %, is given in Table 6, in addition to the more probable value of 7.16 %, on the assumption that 49.15 % of the sweet tasters among the school pupils were missed.

The assumption of a frequency of 7.16 per cent gives better agreement between the observed frequencies of tables 4 and 5 and expected frequencies for sibs in Table 6. This also holds for parents in Table 8, which does not include non-sweet tasters. The lower as well as the higher assumed frequency of sweet tasters in the general population fits the hypothesis that the trait is due to a dominant, autosomal gene.

No observations suggested environmental factors as likely causes of the familial occurrence of sweet tasting for P. T. C. The family material included smokers and non-smokers, teetotallers and moderate to fairly heavy drinkers, one vegetarian family, no extreme food faddists; a predilection for mushrooms was possibly somewhat overrepresented in this series, but otherwise no unusual fondness for any particular dish or drink was observed.

It is difficult to say to what extent the results might have been impaired by the inability of some subjects to describe what they tasted. This may have reduced the frequency of sweet tasters in the first stage of the investigation. This source of error seems to have been eliminated from the second stage of the investigation, *i. e.* the examination of families. At least all persons examined at that stage could distinguish between sweet and bitter. The use of several solutions of various tastes and of distilled water for blind tests reduced the risks of misleading statements to practically nil.

It is apparent from Fig. 1 that refinement of the research technique at the first stage would require at least a wider choice of test solutions. At the second stage assortment technique, *i. e.* distinction of 4 "sweet" solutions from 4 non-sweet solutions would enhance the reliability of the information obtained. The range of sweet tasting of some persons on the scale of P. T. C. solutions (Fig. 1) varied to some extent. This was

observed when retesting probands. Again a source of error is the lack of repeated examinations. It is wellknown that taste acuity varies from time to time. Judging from the present investigation, however, such variation may affect the range but not the presence of the range of sweet tasting.

In view of these sources of error it is not yet possible to regard the hypothesis of autosomal dominance for a gene causing sweet tasting for P.T.C. as established, even though this hypothesis seems more likely than any alternative interpretation including a simple mode of inheritance.

The possibility of a non-random association between sweet tasting for P. T. C. and for quinine sulphate might be suggested by Appendix B, which gives all sweet tasters for quinine sulphate unless they were also sweet tasters for P. T. C. Though quinine sulphate is widely used clinically to test bitter taste discrimination some people taste this substance as sweet. BLAKESLEE and SALMON (1935) observed 2 persons of 47 to taste quinine as sweet in subliminal solutions, in the control series of the present study 3 out of 22 persons tasted solutions of quinine sulphate as sweet. Thus sweet tasters for quinine sulphate dilutions may be fairly common in the general population and the apparently frequent occurence of such sweet tasters in the present family series might be, entirely or partly, coincidental.

SUMMARY

1482 South Swedish school children aged 14 to 21 were examined with P. T. C. solutions 1, 7 and 10 (using the nomenclature of HARRIS and KALMUS, 1949). 54 or 3.64 % were sweet tasters for at least one of these solutions. The frequency of sweet tasters and non-sweet tasters did not vary with sex.

Retesting of 37 probands and examination of their parents and sibs with a complete series of dilutions of P.T.C., together with a test battery of 7 different compounds revealed that the prevalence of sweet tasters for P. T. C. in the general population would most likely be about twice the observed frequency, *i. e.* about 7 %.

The frequency of sweet tasters for P. T. C. among parents and sibs of the probands was compatible with the trait being due to a dominant, autosomal gene.

General parageusia was never observed, but weak concentrations of bitter compounds, including quinine sulphate, were sometimes described as sweet.

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Appendix A. *Families o*

Asterisks refer to probands. The numbers refer to P. T. C. solutions, the letter
before, after or simultaneous wit

Family number	Ref. no of ind. in pedigree	Sex	Age	Taste quality for P. T. C.					Taste quality for quinine sulphate					Taste str		
				O	S	S & B	B	Other sens.	O	S	S & B	B	Other sens.	O	S	S
A I	I 1	M	56	8—13			—7		a			b				
	I 2	F	57	13	12		—11		ab			c				
	II 1	F	22		11—13		—10					a				
	II 2*	F	15	4—5	6—13		—3		ab			c				
A II	I 1	M														
	I 2	F	37	10—13			—9			a		b				
	II 1*	F	15	13	3—12		—2		a	b		c			a	
	II 2	F	13	12—13			—11			a		b		ab		
B I	I 1	M	46	7—13			—3	4—6	ab			c		ab		
	I 2	F	39	1—13					abc					ab		
	II 1*	M	17	7—13	2—6		1		a			b		a	b	
	II 2	M	13	13	5—10		—4	11—12				a				
	II 3	M	12	2—13			1					a				
	II 4	F	8	11—13	8—10		—7					a				
B II	I 1	M	52	11—13	10		—9					a			a	
	I 2	F	44	12—13			—11					a				
	II 1	M	19	12—13	9—11		—8				ab	c			a	
	II 2*	M	14	13	10—12		—9					a				
	II 3*	M	14	13	10		—9	11—12				a				
	II 4	F	9	12—13			—6	7—11				a				
B III	I 1	M	50	12—13			—9	10—11	a			b				
	I 2	F	48	13			—10	11—12	a			b				
	II 1*	M	18	13	11—12	10	—9		a	b		c			ab	
	II 2	M	15				—11	12—13	a		b	c				
	II 3	M	10													
B IV	I 1	M	53	12—13		9—11	—8					a				
	I 2	F	53	13		9—12	—8				a	b				
	II 1*	F	16	12—13		7—9	—6	10—11				a				

weet tasters for P. T. C.

efer to solutions in Table 1. O means tasteless, S sweet taste, S & B means sweet, bitter taste and B means bitter taste.

Taste quality for strychnine					Taste quality for allylthiourea					Remarks
O	S	S&B	B	Other sens.	O	S	S&B	B	Other sens.	
			a					a		
			a					a		NaCl sol. a sweet
			a					a		NaCl sol. a sweet
			a		a			b		HCl sol. a and b sweet sour
										Not examined, said to taste tonicwater as sweet
			a		a					
	a		b		a					NaCl sol. b sweet salty
ab			c		a					NaCl sol. a sweet, sol. b sweet salty
ab			c		abc					P. T. C. sol. 4—6 indefinite
ab			c		ab	c				
a	b		c		b	ac				
			c	ab	c		a		b	P. T. C. sol. 11—12 bitter salty. Allylthiourea sol. b and strychnine sol. a and b salty
			a		c	ab				
			a			a		b		
	a		b			abc				NaCl sol. a and b sweet
			a		a			b		
	a	b	c				abc			NaCl sol a sweet
		a	b				ab		c	Allylthiourea sol. c sweet sour
			a				c		ab	P. T. C. sol. 11—12 indefinite
										Allylthiourea sol. a and b sour
			b	a					abc	P. T. C. sol. 7 bitter salt
										P. T. C. sol. 8—11 salt
										Allylthiourea sol. and strychnine sol. a sour salt
			a			a	b	c		P. T. C. sol. 10—11 indefinite
			a					a		P. T. C. sol. 11—12 indefinite
	ab		c			ab		c		NaCl sol. b sweet
		a	b					a		P. T. C. sol. 12—13 indefinite
										Not examined
			a				ab	c		
		a	b				abc			
			b	a			ab	c		P. T. C. sol. 10—11 bitter + sweet or salt. Strychnine sol. a salt bitter

Appendix A (cont.) 2.

Family number	Ref. no. of ind. in pedigree	Sex	Age	Taste quality for P. T. C.					Taste quality for quinine sulphate				
				O	S	S & B	B	Other sens.	O	S	S & B	B	Other sens.
B V	I 1	M	46	4—13			—3		ab			c	
	I 2	F	45		13	12	—11			a		b	
	II 1*	F	18	13	10—12	6—9	—5			a	b	c	
	II 2	F	13	13	12		—11			a		b	
B VI	I 1	M	36	10—13			—3	4—9			b	ac	
	I 2	F	38	12—13			—10	11	a	b		c	
	II 1*	M	16	11—13		10	—7	8—9				a	
	II 2	M	13	13		11—12	—10			ab		c	
B VII	I 1	M	47		12—13	10—11	—9		a		b	c	
	I 2	F	48	9—13			—8				a	b	
	II 1	F	18	12—13			—11				ab	c	
	II 2*	M	16	13		11—12	—10			a	bc		
	II 3	M	15			10—13	—9			a	bc		
	II 4	M	13	10—13			—9				a	b	
	II 5	M	9	12—13			—11		a			b	
C I	I 1	M	46	8—13		6—7	—5				ab	c	
	I 2	F	44		12—13		—11					a	
	II 1*	F	16	12—13	10—11		—9					a	
	II 2	F	13	12—13			—11		a			b	
C II	I 1	M	43	10—13			—9					a	
	I 2	F	40	11—13		7—10	—6				ab	c	
	II 1*	F	16	13		9—12	—8					a	
	II 2	M	14	13			—12		a			b	
	II 3	M	11	12—13		10—11	—9					a	
C III	I 1	M	40	10—13	3—9	2	1			a		b	
	I 2	F	44	7—13	5—6	2—4	1			a	b	c	
	II 1*	M	15	13	10—12	3—9	—2			a		b	
	II 2	M	12	12—13	7—11	3—6	—2			ab		c	
C IV	I 1	M	48	13			—11	12	b	a		c	
	I 2	F	42	12—13			—11		ab			c	
	II 1*	M	16	13		7—12	—6			a		c	b
	II 2	M	13	11—13		10	—9					a	

(cont.) 2.

Taste quality for strychnine					Taste quality for allylthiourea					Remarks
O	S	S & B	B	Other sens.	O	S	S & B	B	Other sens.	
a			b					a		
			a					a		
		ab	c				ab	c		
										Not examined for strychnine and allylthiourea
		a	b			ab	c			P. T. C. sol. 4—9 indefinite sweet?
										NaCl sol. a salt and sweet
			a					b	a	P. T. C. sol. 11 bitter and sweet?
										Allylthiourea sol. a indefinite
			a					a		P. T. C. sol. 8—9 indefinite sweet?
	a		b					a		NaCl sol. a sweet
	ab		c				a	b		NaCl sol. a and b sweet. HCL sol. a sour sweet
		a	b					a		
		ab	c				a	b		
		a	b				ab	c		P. T. C. sol. 10 sweet at the first stage
		abc					a	b		
		a	b					a		
			a		a			b		
			a			a		b		
			a			ab		c		
			a		a			b		
a			b					a		
			a		ab			c		
		ab	c			a	b			
		ab	c				ab	c		NaCl sol. a sweet
			a					a		
			a				abc			
			a		b	a		c		
		a	b			abc				NaCl sol. a sweet
	a	b	c		a	c	b			NaCl sol. a sweet
		a	b			a	bc			
		a	b		ab			c		P. T. C. sol. 12 sweet?
			a		ab			c		
			a		ab			c		
			a		a			c	b	Allylthiourea sol. b indefinite

Appendix A (cont.) 3.

Family number	Ref. no of ind. in pedigree	Sex	Age	Taste quality for P. T. C.					Taste quality for quinine sulphate				
				O	S	S & B	B	Other sens.	O	S	S & B	B	Other sens.
C V	I 1	M	52	13			—12		ab			c	
	I 2	F	46	13	12	9—11	—8			a	b	c	
	II 1*	M	18	12—13		10—11	—9			a	b	c	
	II 2	M	14		11—13		—10		a			b	
C VI	I 1	M	54	9—13			—8					a	
	I 2	F	50	13		12	—11		a			b	
	II 1*	F	15		13	8—12	—7				a	a	
C VII	I 1	M	42	9—13			—8		a				bc
	I 2	F	43	3—13			1	2	a	b		c	
	II 1*	F	15		5—13		—4			ab		c	
	II 2	F	9	13	12		—10	11				a	
	II 3	M	7	9—13			—8		a			b	
C VIII	I 1	M	41	12—13			—9	10—11	ab	c			
	I 2	F	40	10—13			—9					a	
	II 1*	F	17	12—13	10—11	9	—8			ab		c	
	II 2	F	10	11—13			—10				ab	c	
C IX	I 1	M	40	11—13	9—10		—8				abc		
	I 2	F	40	10—13			—9		ab			c	
	II 1*	M	15	10—13	8—9	5—7	—4			ab		c	
	II 2	M	14	9—13	8		—7		a		b	c	
	II 3	M	6	12—13			—11		a			b	
D I	I 1	M	75	7—13			—6		ab			c	
	I 2	F	62			13	—12		a		b	c	
	II 1*	M	19		6—13	3—5	—2		ab			c	
D II	I 1	M	52	8—13			—7		a	b		c	
	I 2	F	52			11—13	—10		a			b	
	II 1	M	20	8—13			—7		abc				
	II 2*	M	15		10—13	8—9	—7		a			b	
D III	I 1	M	52	13			—12		ab	c			
	I 2	F	51			12—13	—11		ab			c	
	II 1*	M	16	11—13		9—10	—8		ab			c	

(cont.) 3.

Taste quality for strychnine					Taste quality for allylthiourea					Remarks
O	S	S&B	B	Other sens.	O	S	S&B	B	Other sens.	
a			b					a		
		ab	c			ab		c		NaCl sol. a sweet
		ab	c			ab	c			
	a		b			ab	c			
			a		a			b		
		a	b		a			b		
		ab	c			a		b		
ab			c					a		Quinine sulphate sol. a and b sour
a			b		abc					P. T. C. sol. 2 indefinite
										NaCl sol. a sweet
	ab		c			abc				NaCl sol. a sweet
										P. T. C. sol. 11 indefinite
ab			c		abc					NaCl sol. a sweet
a				bc	b			c	a	P. T. C. sol. 10—11 bitter + sweet or salt. Allylthiourea sol. a and strychnine sol. b salt. Strychnine sol. c bitter and salt
		ab	c					a		
		ab	c				a	b		NaCl sol. a and b sweet. HCL sol. a and b sour sweet
			a			a		b		NaCl sol. a sweet
			a					a		
ab			c		ab			c		
		ab	c			a	b	c		
		a	b		a	b		c		
			a			a		b		
			a					a		
	b	a	c					a		
		ab	c				abc			NaCl sol. a and b sweet
			a		ab			c		
			a					a		
			a		ab		c			
			a		ab			c		
ab			c		a			b		
a			b		a			b		
a			b		ab			c		NaCl sol. a sweet

Appendix A (cont.) 4.

Family number	Ref. no of ind. in pedigree	Sex	Age	Taste quality for P. T. C.					Taste quality for quinine sulphate				
				O	S	S & B	B	Other sens.	O	S	S & B	B	Other sens.
D IV	I 1	M	33	7—13	5—6	4	—3					a	
	I 2	F	33	10—13			—9					a	
	II 1*	M	15		10—13	9	—8		a			b	
	II 2	F	7	8—13			—7		ab			c	
D V	I 1	M	53	10—13			—8	9	a	b		c	
	I 2	F	46	10—13	5—9		—2	3—4		abc			
	II 1	M	22	11—13			—10		ab			c	
	II 2*	M	18	12—13	10—11	9	—8			ab	c		
	II 3	F	13	10—13		9	—8			abc			
D VI	I 1	M											
	I 2	F	44	12—13			—11		ab			c	
	II 1*	M	15	12—13	11	10	—9		a		b	c	
	II 2	F	11	11—13	10		—9		a			b	
D VII	I 1	M	59		10—13	9	—8			a	bc		
	I 2	F	52			12—13	—11			ab		c	
	II 1*	M	21	13	9—12	8	—7			a	bc		
	II 2	F	17			12—13	—11			a	b	c	
D VIII	I 1	M	47	11—13			—9	10	a	b	c		
	I 2	F	47	13		10—12	—9					a	
	II 1*	M	15	2,9—13	3—8		1		ab			c	
	II 2	F	10	13	10—12		—9					a	
D IX	I 1	M	39	10—13	8—9	2—7	1					a	
	I 2	F	38	12—13			—11					a	
	II 1*	M	15	12—13	10—11	9	—8			a	bc		
	II 2	F	13	13	11—12	10	—9				a	b	
D X	I 1	M	47	3—13	2		1		ab			c	
	I 2	F	44	11—13			—10		ab			c	
	II 1*	M	16	10—13	8—9	6—7	—5		b	a		c	
	II 2	M	10	10—13			—9			ab		c	
E I	I 1	M	45	11—13			—10					a	
	I 2	F	43			12—13	—11				a	b	
	II 1*	F	15	10—13	7—9	6	—5			ab	c		
	II 2	M	12	10—13		8—9	—7					a	

(cont.) 4.

Taste quality for strychnine					Taste quality for allylthiourea					Remarks
O	S	S&B	B	Other sens.	O	S	S&B	B	Other sens.	
	a		b					a		NaCl sol. a and b sweet. HCl sol. a sweet
			a					a		
	a		b					a		
a			b		ab			c		
	a	b	c			ab		c		P. T. C. sol. 9 indefinite
		ab	c			ab		c		P. T. C. sol. 3—4 indefinite
										Quinine powder and tonic water reported to taste sweet
			a		ab			c		
	a	b	c			ab	c			Distilled water sweet
			a			ab		c		
										Not examined
a			b					a		
		ab	c		a		b	c		
			a		a		b	c		
	ab		c			ab		c		NaCl sol. b sweet
		ab	c				ab	c		
	ab	c			ab			c		NaCl sol. a and b sweet
	a	b	c			a	b	c		
a	b		c					a		P. T. C. sol. 10 indefinite
			a					a		
a			b		a			b		
			a			a		b		
			a				abc			NaCl sol. a sweet
a			b					a		
	a		b			a	b	c		NaCl sol. a and b sweet
										Distilled water sweet
		a	b				ab	c		
ab			c		ac	b				
ab			c		a			b		
	a		b		a	b			c	Allylthiourea sol. b indefinite
	a		b		abc					
			a					a		
		ab	c					a		
	ab	c				abc				
			a					a		

Appendix A (cont.) 5.

Family number	Ref. no of ind. in pedigree	Sex	Age	Taste quality for P. T. C.					Taste quality for quinine sulphate				
				O	S	S&B	B	Other sens.	O	S	S&B	B	Other sens.
E II	I 1	M	59	11—13			—10		ab			c	
	I 2	F	51	11, 13	12		—10					a	
	II 1*	F	17	10—13	9		—8					a	
	II 2	M	14	10—13	9		—8					a	
E III	I 1	M	46		7—13	3—6	—2			ab	c		
	I 2	F	43	10—13			—9			a	b	c	
	II 1*	F	17	11—13	7—10	6	—5			a		b	
	II 2	F	7	10—13			—9		a	b		c	
E IV	I 1	M											
	I 2	F	48			11—13	—9	10		a	bc		
	II 1*	F	15	12—13	10—11		—9				ab	c	
	II 2	F	13	10—13			—8	9		a	b	c	
	II 3	M	12	11—13			—8	9—10			ab	c	
	II 4	M	7	13	10—12	8—9	—7			a	a	c	
E V	I 1	M											
	I 2	F	40	11—13			—10					a	
	II 1*	F	17	10—13	7—9		—5	6		ab		c	
	II 2	F	16	9—13			—8		ab			c	
	II 3	F	15		11—13	9—10	—8					a	
E VI	I 1	M	53		12—13		—11			a	b	c	
	I 2	F	50	12—13			—11					a	
	II 1*	F	16	13	10—12	6—9	—5				ab	c	
E VII	I 1	M	43	10—13			—9			a	b	c	
	I 2	F	42	8—13			—7		a	b	c		
	II 1*	F	17	13		10	—9	11—12	a			b	
E VIII	I 1	M	58	11—13		9—10	—8		a			b	
	I 2	F	52	11—13			—10		a			b	
	II 1*	F	18	12—13		10—11	—9		a			b	

(cont.) 5.

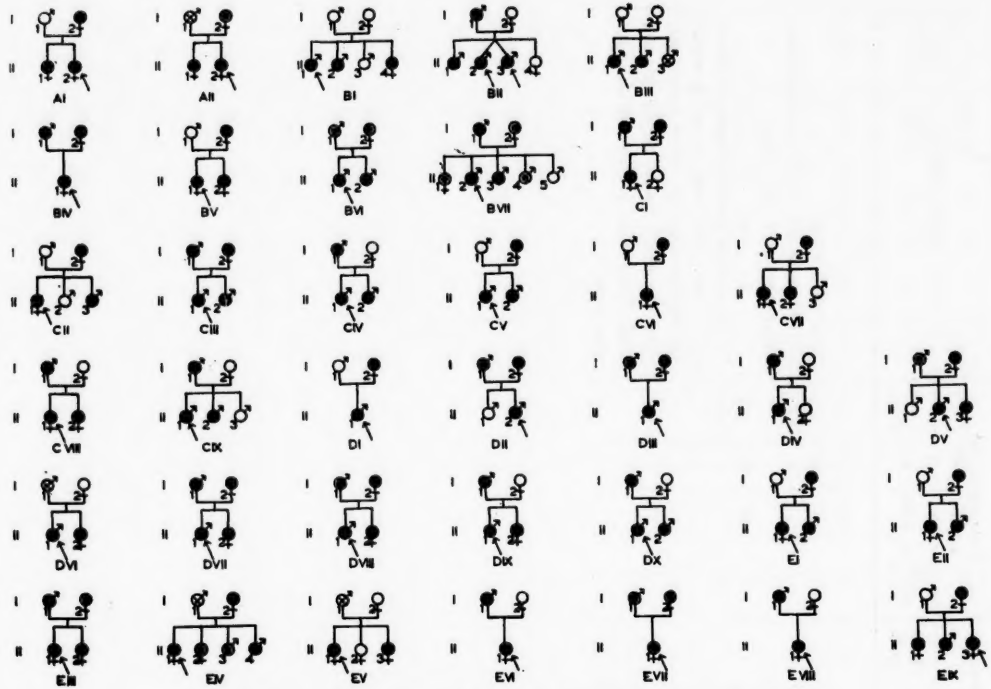
Taste quality for strychnine					Taste quality for allylthiourea					Remarks
O	S	S&B	B	Other sens.	O	S	S&B	B	Other sens.	
a			b					a		
			a					a		
			a					b	a	NaCl sol. a sweet. HCl sol. a sour sweet.
										Allylthiourea sol. a sour sweet
			a					a		NaCl sol. a sweet
	ab		c			ab	c			NaCl sol. a and b sweet. HCl sol. a sweet.
										Distilled water sweet.
a		b	c		a			b		NaCl sol. a and b sweet
			a			abc				
	ab		c			ab		c		
										Not examined
		a	b		a		bc			P. T. C. sol. 10 indefinite
										NaCl sol. b sweet. HCl sol. a sweet
	a		b		a		bc			NaCl sol. b sweet. HCl sol. a sweet. HCl
										sol. b sour sweet
		ab	c		a		bc			P. T. C. sol. 9 indefinite
										NaCl sol. b sweet. HCl sol. a sour sweet
a			b		a		b	c		P. T. C. sol 9—10 indefinite
										NaCl sol. b sweet. HCl sol. a sour sweet
		ab	c		a	bc				NaCl sol. a sweet. HCl sol. a sour sweet
										Not examined
			a					a		
ab			c		abc					P. T. C. sol. 6 indefinite
										HCl sol. a sour sweet
	a		b		ab			c		
		a	b				ab	c		NaCl sol. a and b sweet
		a	b				ab	c		
	a	b	c			a	bc	a		
		ab	c							
			a					a		
ab			c					a		
			a					a		P. T. C. sol. 11—12 indefinite
a			b			a	bc			NaCl sol. b sweet. HCl sol. a and b sour
										sweet
			a					a		
a		b	c				ab	c		

Appendix A (cont.) 6.

Family number	Ref. no of ind. in pedigree	Sex	Age	Taste quality for P. T. C.					Taste quality for quinine sulphate				
				O	S	S&B	B	Other sens.	O	S	S&B	B	Other sens.
E IX	I 1	M	47	13			—12					a	
	I 2	F	45		13	10—12	—9				ab	c	
	II 1	F	21	9—13		8	—7					a	
	II 2	M	20	13		11—12	—10					a	
	II 3*	F	18	12—13		11	—9	10			ab	c	

(cont.) 6.

Taste quality for strychnine					Taste quality for allylthiourea					Remarks
0	S	S&B	B	Other sens.	0	S	S&B	B	Other sens.	
			a					a		
		ab	c				ab	c		NaCl sol. b sweet
			b	a				c	ab	Allylthiourea sol. a and b sour bitter. Strychnine sol. a sour sweet
		ab	c			a	b	c		
			c						abc	P. T. C. sol. 10 sometimes sweet sometimes salt. NaCl sol. a and b sweet All allylthiourea sol. sour bitter, salt bitter or sweet bitter. Strychnine sol. a salt. Strychnine sol. b salt with sweet after taste



Appendix B. *Pedigree cards of the families of sweet tasters.*

○ non-sweet taster for P. T. C. ◉ non-sweet taster for P. T. C and sweet taster for quinine-sulphate
● sweet taster for P. T. C. ⊗ not examined

BEHAVIOUR OF DIFFERENT SMALL ACCESSORY RYE CHROMOSOMES AT POLLEN MITOSIS

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INTRODUCTION

SEVERAL types of accessory chromosomes are known in rye, the majority of which are considerably smaller than the standard accessory. Two, the small iso-accessory and the deleted accessory, have been more closely investigated by MÜNTZING. Through crosses it was shown that the two chromosome types have lost the peculiar property of lagging and non-disjunction to the generative pole at the first mitosis of the pollen cell which distinguishes the standard accessory. This was also verified cytologically (MÜNTZING 1946, 1948). The small iso-accessory is derived from the short arm of the standard accessory whereas the deleted accessory has lost the distal part of its long arm (see MÜNTZING 1958).

Here I shall describe observations on pollen mitosis in plants with a small chromosome and also in others with two standard and one or more small accessories. Small accessories are rarely observed at pollen mitosis. MÜNTZING (1946) found in a plant with the small iso-accessory among a total of 54 metaphases only 4 with the small accessory, at anaphase it was only observed dividing in 7 of 151 counted cells.

14+1 SMALL ACCESSORY

The investigated plants belonged to rye from Korea which has a considerably higher frequence of accessory chromosomes than other investigated types (MÜNTZING 1957). Cytological investigations of this rye have not been made previously. Judging from root mitosis the accessory had a median centrometre in one or two plants; in two plants the centromere was seemingly not median and in one plant the small

chromosome seemed to be a ring chromosome. In all, five plants were investigated but only in two plants was the behaviour of the small chromosome observed at pollen anaphase.

It is generally more easy to find the small chromosome in prometaphase than later at anaphase. Clearly a strong elimination at meiosis had occurred, for only in a rather small minority of pollen cells is the small chromosome detected. In several plants a small nucleolus was secreted in the cytoplasm from one or both anaphase groups of chromosomes, and several still smaller nucleoli may sometimes occur. At late anaphase the spindle in several plants was often strongly bent.

In 043-107 the small chromosome was comparatively often observed at prometaphase. Once two such chromosomes were observed (Fig. 1). This indicates an exceptionally low degree of meiotic elimination. At anaphase no lagging was observed but in four pollen cells the division of the small chromosome was observed (Figs. 2, 3), this phenomenon being once seen in both anaphase plates in polar view (Fig. 4).

In 043-436 the accessory chromosome seemed somewhat smaller than in 043-107. Usually it had a peripheral position at prometaphase and metaphase and is somewhat later arranged in the plate than A chromosomes. At anaphase it was difficult to observe the small chromosome. However, lagging and non-disjunction may clearly occur (Fig. 5). In three cells the small chromosome was observed in the generative pole group whereas it was absent from the inner group.

Moreover, in two plants, 042-4 and 043-180, a number of anaphase stages were observed. Division of the small chromosome seemed probable but no certain case of lagging or division was observed. The ring chromosome in plant 043-408 was not observed at any anaphase.

14+2 st+SMALL ACCESSORIES

Pollen mitosis of Östgöta gråråg plants with two standard and one to three small accessories was investigated. In view of certain results of BOSEMARK, who has investigated accessory chromosomes in *Festuca pratensis* (BOSEMARK 1956), a changed behaviour of small accessories was not improbable in such plants.

The plant 11—10 had 2 standard and 2 deleted accessories. A normal bipolar spindle was as rule formed. The small accessory had generally a position near the centre of the plate. The same position has been established by MÜNTZING in root mitosis (MÜNTZING 1948). At anaphase the small accessory was, as expected, more rarely seen whereas the

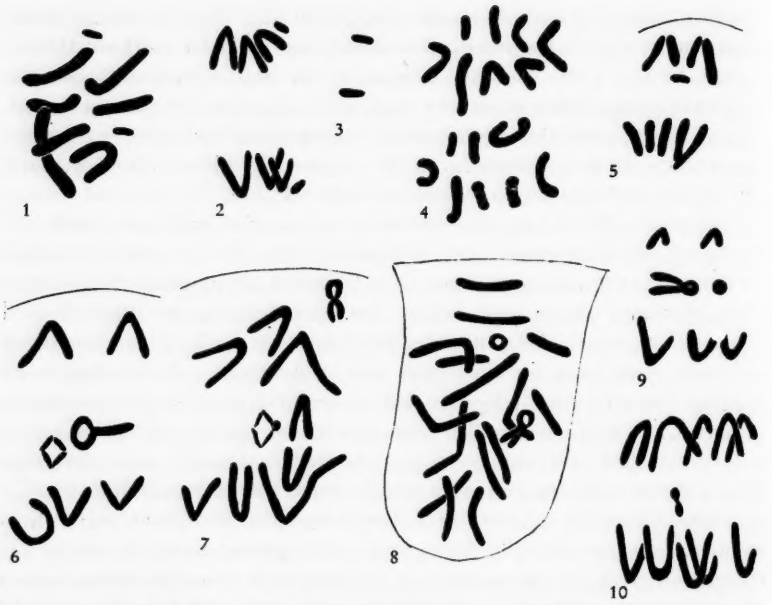
standard accessory was observed to lag quite often. Fig. 6 shows the small accessory as well as the standard accessory lagging between the anaphase groups. In Fig. 7 the standard is lying at the generative pole above the anaphase group. Other cases of a lagging small accessory were observed.

In observed cases the lagging small chromosome had a fairly constant appearance. It was rhombiform, its chromatids being united at each end: a sort of knob may be visible at one end (Fig. 7).

The plants 026-3 had two standard accessories and one small iso-accessory. Prometaphase and metaphase were fairly often disturbed here, having similar appearance as is frequent in rye plants with six or more standard accessories, which have been profusely illustrated in two earlier papers (HÅKANSSON 1948, 1957). It looks like a modified c-mitosis with long chromosomes and a deficient spindle sometimes causing three or four telophase nuclei and frequent bridge formation (Figs. 11, 12). Disturbed pollen mitoses with one small and two standard accessories and two small and one standard accessory were observed. Fig. 8 shows a pollen cell with widely separated A chromatids having no connection with spindle fibres; the chromatids of the accessory chromosomes are not completely separate, thus giving these chromosomes their characteristic appearance. 15 A chromatids could be distinguished — perhaps the cell was trisomic — one chromatid being present in another section. The small accessory was often a ring.

More often, however, the spindle was bipolar. There were sometimes one or two chromosomes outside the plate. Only rarely could the small iso-chromosome be observed at anaphase, but the lagging standard was more often seen. Presumably most pollen cells with standard and small accessories have disturbed mitosis not forming a bipolar spindle. In Fig. 9 standard and small accessories lag. Once or twice an accessory seemed to degenerate between the anaphase groups. Fig. 10 shows the small accessory lagging although it is not accompanied by a standard accessory. To observe this accessory lagging in a cell which has no standard accessory was not expected, but too much weight can hardly be attached to a single observation.

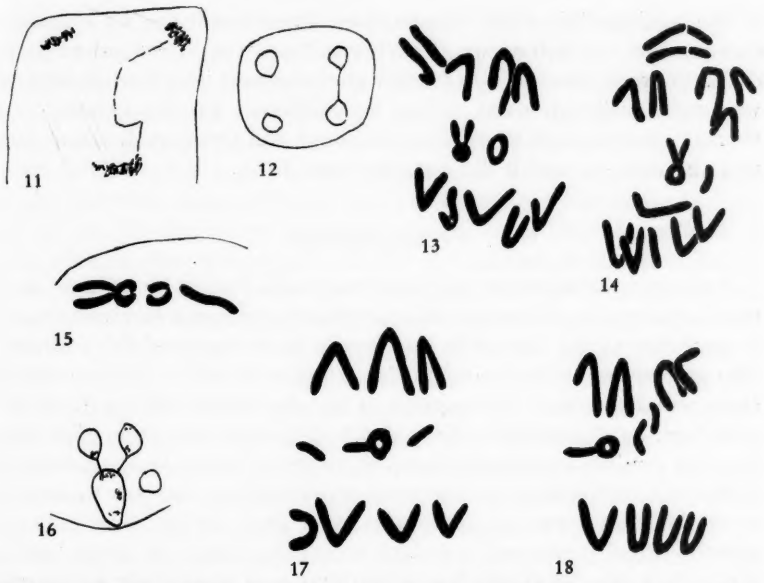
017-10 had two standard and three small chromosomes. A comparatively small number of loculi with pollen mitosis were investigated. Two small and one standard accessory were several times observed. The division was then disturbed in the way described above. Once a regular anaphase was shown by only one chromatid pair. A bipolar spindle, however, was much more frequent, and in four pollen cells a small chromosome as well as a standard accessory were lagging. The small



Figs. 1—4. Plant 043-107. — Fig. 1, late prometaphase, 2 small accessories. — Fig. 2, anaphase, small accessory divided. — Fig. 3, divided small accessory at anaphase. — Fig. 4, a small accessory is visible in the two anaphase plates. — Fig. 5. Plant 043-436. Anaphase, the small chromosome shows non-disjunction to the generative pole. — Figs. 6—7. Plant 11—10, anaphase. Small and standard accessory lagging. Fig. 7 compare the text. — Figs. 8—11. Plant 026-3. Fig. 8: upset pollen mitosis. — Fig. 9, anaphase, standard and small accessory lagging. — Fig. 10, anaphase, the small chromosome lags.

chromosome was at this stage similar to a ring (Fig. 13). It was also observed nearer the generative pole. Often chromosomes were in an abnormal position on the spindle, when both a small and a standard accessory were present (Fig. 14).

7—8 had two standard and three small chromosomes. A larger number of slides could be investigated here. In the pollen cells 0, 1 or 2 small chromosomes were observed. The c-mitotic disturbance was rather frequent here, the small accessories usually forming rings. A bipolar spindle was, however, more frequent and the standard accessory was often observed lagging. On only a few occasions was the small chromosome also lagging. It had a similar appearance to that in 17—10. It was also observed nearer the generative group and certainly goes to the



Figs. 11—12. Plant 026-3. More than two telophase nuclei were formed. — Figs. 13—14. Plant 17-10. Anaphase, small and standard accessory lagging. A chromosomes with curious positions occur. — Figs. 15—16. Plant 7-8. Fig. 15, small and standard accessories as well as an A chromosome lag near the generative pole. — Fig. 16, a curious telophase after upset mitosis. — Figs. 17—28. Plant 045-9. Fig. 17, standard accessory and two small accessories lagging. — Fig. 18, the small accessories move to the generative pole.

generative pole. Fig. 15 shows lagging of the accessory chromosomes and an A-chromosome. Fig. 16 a curious telophase.

045-9 also had two standard and three small chromosomes. The small chromosome was a new accessory and deviated in several respects. One or two mere frequently observed — also three, but this rarely — in the pollen cells. In contradistinction to 7—8 and 017-10, which also had three small chromosomes, c-mitosis was very rarely observed. Only exceptionally are chromosomes observed outside or in an abnormal position on the anaphase spindle here. As a rule the small chromosomes had a longitudinal position. It goes to the generative pole in this position (Fig. 18). The transversal position in the spindle which was shown in other investigated plants was only once observed in 045-9. The centromere is probably more terminal here, though there also seems to be a median constriction in the small chromosome.

The lagging of the small chromosome shows that it was an accessory chromosome. Its behaviour at pollen mitosis was in several respects different from those of other small chromosomes investigated here. It was more easily observed, it had less influence on the spindle — a normal metaphase plate with one standard and three small accessories was observed —, and it did not show ring form.

CONCLUSIONS

Accessory chromosomes are common in the family *Gramineae*. In a number of species numerical increase of the accessories has been found. A mechanism only known in this family is the cause of this increase. The accessories at first — or in *Zea mays* at second — pollen mitosis show non-disjunction. At anaphase of the first pollen mitosis the accessory lags, its chromatids adhere and both pass to the generative pole. In other families investigated cases of numerical increase are caused by a directed distribution of univalent accessories at meiosis. In several cases different types of accessories have been found: they may be morphologically changed standard accessories, most of them having diminished size. MÜNTZING has shown that such smaller rye accessories have lost the property of non-disjunction; and BOSEMARK (1956) has made similar observations in *Festuca pratensis*. The changed behaviour of the small accessories is no doubt due to the loss of a certain part of the standard accessory. Pachytene studies by MÜNTZING and LIMA-DE-FARIA showed that the deleted accessory har lost the distal part of the long arm. A heterochromatic knob near the end was assumed responsible. BOSEMARK as a second factor assumes changes of the centromere, finding in *Festuca pratensis* two very similar chromosomes of diminished size, one showing, the other lacking non-disjunction.

Investigations on Korea rye have revealed new cases of small rye accessories. In strain 043 the chromosomes had been counted in 436 plants, and in five plants there was a small accessory. A small accessory with non-disjunction was found in the plant 043-436. Presumably this chromosome had not lost the important chromosome segment, which is absent in other small accessories. Small chromosomes with non-disjunction have not previously been found in rye.

BOSEMARK (1956) investigated pollen mitosis in plants with accessories of diminished size as well as standard accessories. Concerning the plant 2311 he writes "it is very likely that the presence of standard accessory chromosomes in pollen grains is essential for a successfull

non-disjunction of this small chromosome". The small chromosome of plant 373, on the other hand, increased the proportion of non-disjunction of the standard accessory, the same effect on the standard accessory being produced by the large iso-accessory. Different accessory chromosomes evidently influence each other at pollen mitosis.

These interesting results of BOSEMARK were mostly attained through crosses between plants with two kinds of accessories and plants lacking accessories. In the present case a cytological study of pollen mitosis in such plants in *Secale* was made. In five investigated plants the standard as well as the small accessory showed lagging, and thus non-disjunction. It is known that deleted and small iso-accessories show non-disjunction when a standard chromosome is present. Thus the accessories show a similar behaviour as in *Festuca*, the standard accessory forcing the small accessories to undergo non-disjunction. The small rye accessories are comparatively smaller than in *Festuca pratensis* and an influence in reversed direction from the small to the standard accessory causing a lowered frequency of non-disjunction does not seem to occur. This question, however, can only be decided through adequate crosses.

The standard accessory has a constant appearance after splitting. The chromatids are united in a segment in the proximal part of the long arm. In a lesser degree the chromatids adhere at the end of the short arm. On the other hand, lagging small chromosomes with one exception often had ringform, the chromatids seeming to adhere at both ends. In c-mitosis this is clearly seen.

In several plants with small and standard accessories pollen mitosis was disturbed. One standard accessory does not disturb pollen mitosis (HÅKANSSON 1948). Thus the small accessory has a similar influence as the standard. In plants with two standard and three small accessories pollen mitosis was disturbed fairly often, recalling the division in plants with six accessories. In pollen cells with one standard and one small accessory the plate when formed was often incomplete. In 026-3 the disturbance was also observed though in this plant there was only one small accessory. It had, however, the small arm duplicated and this may perhaps be the explanation. On the other hand 045-9 with three small accessories showed no or only slight disturbance. The small chromosomes here showed somewhat different properties and apparently largely have lost the property of disturbing the spindle. The standard accessory had no possibility to influence the small chromosome in this respect. Pollen mitosis was also strongly disturbed in a plant with three standard and three small accessories described earlier (HÅKANSSON

1957). The behaviour of the small accessories at anaphase was not, however, clarified in this plant.

It was suggested that the rye accessory does not give an adequate contribution to the forming of a normal spindle, the latter being abnormal when three accessories are present in the pollen cell (HÅKANSSON 1957). This may be a gene effect as is assumed by ÖSTERGREN (1947) concerning non-disjunction through genes located in the same chromosome. MÜNTZING has stressed the importance of a certain heterochromatic part of the chromosome, a knob near the distal end of the long arm; and an effect of heterochromatin in similar non-disjunction has also been shown for *Zea mays* (LONGLEY 1956) and *Festuca pratensis*. In the latter species BOSEMARK also assumed that changes in the centromere had occurred. It seems that the factors disturbing the spindle in rye are situated in another part of the standard accessory and a location to the small arm is indicated. The new small accessory in 045-9 may mainly consist of part of the long arm.

SUMMARY

Pollen mitosis in Korea rye with one small accessory chromosome showed at anaphase in one plant non-disjunction, in a second plant division of these chromosomes.

In plants of Östgöta gråråg with two standard and one to three small accessories not only the standard but also the small accessories show non-disjunction at pollen anaphase. The standard accessory influences the small accessories causing them to lag.

Most small accessories may disturb the spindle in the same degree as the standard accessory.

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WITH SPECIAL REFERENCE TO TRITIUM LABELED DNA PRECURSORS

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IN the last decade autoradiography has gone through a very rapid development and its methods have continuously improved. The use of P^{32}, C^{14}, S^{35}, I^{131} and other radioisotopes, in connection with stripping film and liquid emulsion techniques, has proven a most valuable tool in the study of cell metabolism and nuclear function. The introduction of tritium, with its low energy beta particles, has made the technique applicable to analysis of single chromosomes and chromosome segments.

In a short period of time the use of tritium labeled DNA and RNA precursors has led to the accumulation of a large body of information on chromosome organization. Significant information has been obtained on: (1) DNA replication in mitotic chromosomes, (2) synthesis of DNA and metabolism of RNA in puffs of giant chromosomes, (3) uptake of RNA precursors in the nucleolus and other cell components, (4) sequence of replication of DNA and basic protein in the macronucleus of *Euplotes*, (5) the differential uptake of tritiated thymidine by hetero- and euchromatin in meiotic chromosomes.

Like all other methods, the technique contains its limitations. As a result controversy has arisen on occasion. For certain problems the use of this technique in combination with specific chemical or enzymatic extraction and/or photometric measurements becomes desirable or necessary.

Autoradiography has revealed itself an equally valuable tool in the study of: (1) histochemistry in relation to metabolic disorders and cancer, (2) tissue transplantation, (3) uptake of radioisotopes by bone, (4) nucleic acid metabolism, (5) relation between nucleic acid and protein synthesis, (6) DNA replication in phage, (7) cell turnover in hemopoietic tissues, (8) DNA metabolism in human cancer cell cultures, (9) renewal and migration of cell populations, and in many other fields.

The bibliography includes not only papers dealing with the problems referred to above, but also those dealing with various aspects of the technique, such as: liquid scintillation counting, grain counting instruments, performance of stripping emulsions, methods of labeling, types of emulsion, health physics considerations, and others.

The main emphasis has been placed on autoradiography with tritium labeled compounds, since this is the author's field of work.

Three Symposia have been published on problems dealing mainly with tritium labeled compounds and autoradiography:

(1) Symposium on tritium in tracer applications. 1957. — New England Nuclear Corp. New York.
(2) Symposium on advances in tracer applications of tritium. 1958. — New England Nuclear Corp. New York.
(3) Conference on autoradiography. 1959. — Laboratory Investigation *8*: 59.

The following papers may be considered as principal sources of reference for the technique.

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THE MITOTIC CHROMOSOMES OF SOME CAVICORN MAMMALS (BOS TAURUS L., BISON BONASUS L. AND OVIS ARIES L.)

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I. INTRODUCTION

THE recent improvement in the technique of mammal chromosome study permits the addition of new details to our knowledge and makes reinvestigations desirable in some cases where many chromosome investigations are already published as regards cattle, goat, pig and horse among the common domestic mammals.

The relatives of domestic mammals are often imperfectly known or indeed unknown from a chromosomal point of view but it can be expected that sufficient primary datas will by and by be available, making possible well-founded conclusions about the chromosomal situation not only in the domestic mammals themselves but also in the taxonomic units to which they belong.

This paper contributes to the morphology of mitotic chromosomes in some species of the family *Bovidae* (*Cavicornia*). The species are: Cattle (*Bos taurus*), European bison or wisent (*Bison bonasus*) and sheep (*Ovis aries*).

Owing to the relative inacessibility of these large animals I have made the chromosome preparations when suitable material occasionally came to hand. The cattle and sheep cells originate from lung tissue of about half-grown embryos, the exact age of which was not obtainable as the material was collected at a slaughterhouse. The age of the cattle embryos was estimated to be about four months and that of the sheep to be about two months.

The chromosome preparations were orcein squashes (1 % orcein in 60 % acetic acid). As a basis of the idiogram analyses every single chromosome was separately drawn in the centre of the view field of the microscope at $\times 3700$, using a camera lucida.

II. EUROPEAN DOMESTIC CATTLE, BOS TAURUS L.

Previous works

Since the work of BARDELEBEN 1892 many papers have been published on the chromosomes of European domestic cattle (SHOENFELD 1902, VAN HOOF 1919, MASUI 1919, WODSEDALEK 1920) reporting a chromosome number ranging from 16 to 38. KRALLINGER (1927, 1928) was the first one to report 60 chromosomes in this species, a number that has been confirmed by later workers (MAKINO 1944, MELANDER and KNUDSEN 1953, KNUDSEN 1954, LEUCHTENBERGER *et al.* 1956, POSTIGLIONI-GRIMALDI 1957, MELANDER 1957). The earlier deviating reports may be ascribed to technical difficulties when treating the material.

Own observations

The material of the European domestic cattle comes from the Swedish breed S.L.B., a descendant of the Dutch lowland (Holstein breed). MAKINO used the same breed for his work (1944).

The diploid chromosome number has been found to be 60 in more than 200 cells in full metaphase when only plates with an unequivocally undisturbed condition were counted (Figs. 1 a—c; 2 b; 4; 5).

58 chromosomes, the autosomes, have a strictly terminally located centromere. The X is submedially inserted and belongs to the larger chromosomes. The Y is among the smallest ones and is submedially inserted (Figs. 1 a, b; 2 b; 3; 4). It is always found to be positively heteropycnotic at metaphase in my material (Fig. 1 d). The length of the chromosomes ranges from about 4 to 1 micra. No secondary constrictions have been observed.

I have paid special attention to the morphology of the sex chromosomes since KRALLINGER (1931) pointed out an unpaired V-shaped chromosome as being the X. This was denied by MAKINO (1944), who could find no evidence for the existence of any V-formed chromosome in the cattle complement. He claimed that all the chromosomes of this species are rod-shaped. Dealing with the shape of the Y-chromosome MAKINO says that "the Y seems to be the one a little longer than the smaller autosomal elements". Furthermore, he hesitates to point out the sex chromosomes in the diploid complex.

With the technique applied in the present investigation both sex chromosomes can be identified at mitosis owing to the median location of

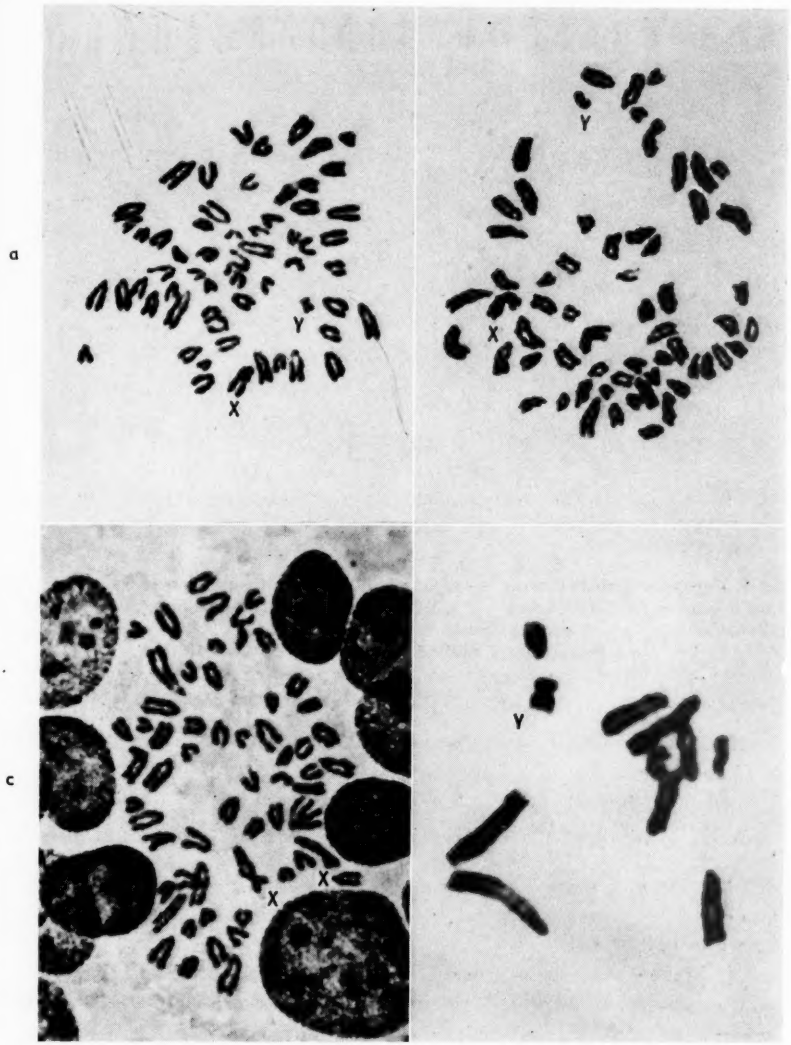

Fig. 1. European domestic cattle. — a—b. Mitotic plates from two bulls of the S.L.B-breed. — ×1250. — c. Mitotic plate from a cow of the S.L.B-breed. — ×1250. — d. Part of a mitotic metaphase plate from a bull of the S.L.B.-breed showing the medially inserted, positively heteropycnotic Y. — ×2500.

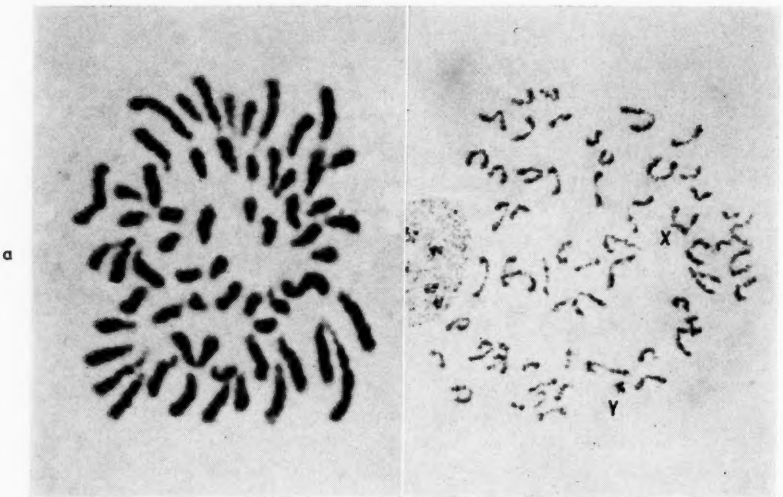

Fig. 2. European domestic cattle. — a. One half of a mitotic anaphase in polar view from a bull of the S.L.B.-breed. — ×2500. Untreated. The centromeres are located quite terminally, which can be clearly seen in some cases. — b. Early telophase in a c-mitosis from a bull of the S.L.B.-breed. — ×1250.

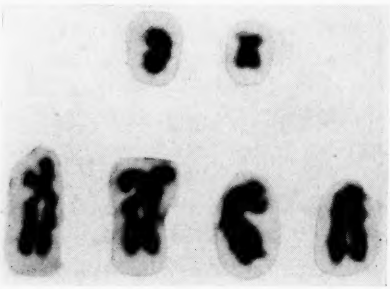

Fig. 3. European domestic cattle. — Four X- and two Y-chromosomes from mitotic plates of the S.L.B.-breed. — ×3000.

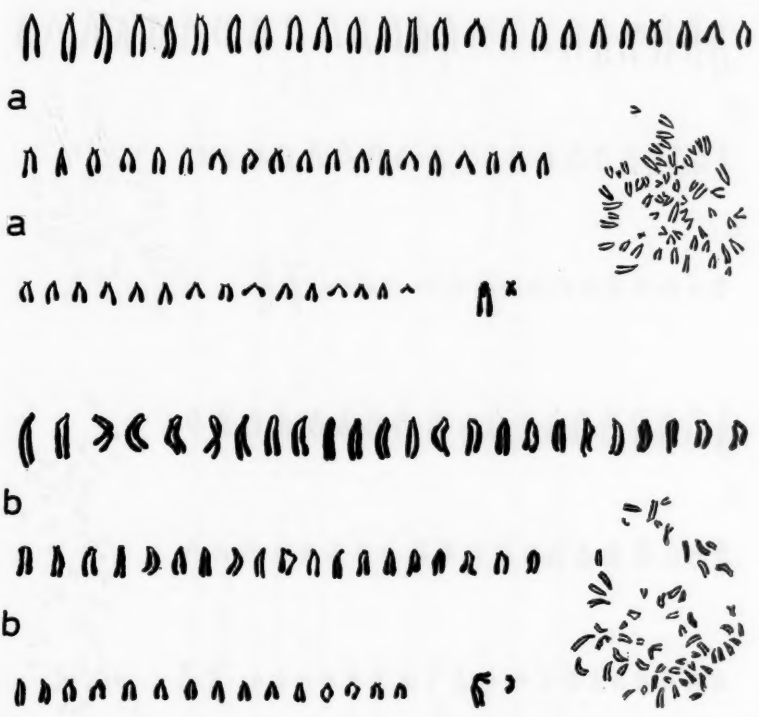

Fig. 4. European domestic cattle. — Idiograms from two bulls of the S.L.B.-breed.

their centromeres (Figs. 1 a, b; 2 b; 3; 4). To my knowledge it is unique among mammals that both sex chromosomes can be so easily and definitely distinguished at mitosis.

III. EUROPEAN BISON OR WISENT, BISON BONASUS L.

Thanks to the courtesy of K. CURRY-LINDAHL, director of The Skansen Zoological Gardens in Stockholm, I obtained material for chromosomal study of the 25-year old wisent cow Birma (called 226 Pustelnica in the herd book) when she was put down in October 1957.

The wisent is a species in extinction. Apparently there are no wild animals left today. The number of pure-bred specimens in captivity in 1959 is estimated at about 280—300 by E. MOHR (pers. com.).

As far as I can find nothing is published about the chromosomes of

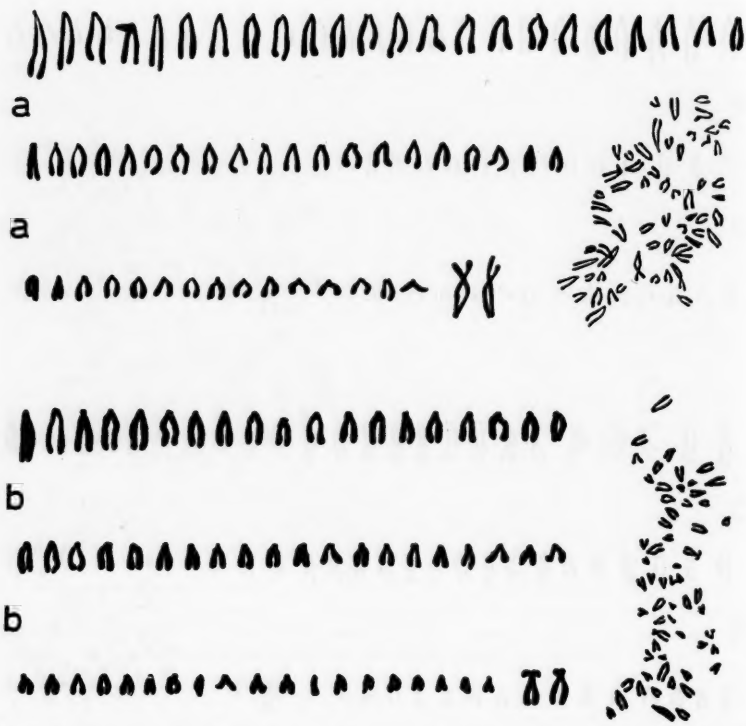

Fig. 5. European domestic cattle. — Idiograms from two cows of the S.L. B.-breed.

the wisent. The chromosome number of the wisent is 2n=60. The 58 autosomes have their centromeres situated completely terminally. The X-chromosome, which (if analogous with that of cattle) is among the longest ones, has a submedially located centromere (Figs. 6; 7). In one plate the length of the chromosomes ranges from 5.7 to 1.5 micra. No secondary constrictions have been observed.

I am unable to point to any morphological differences between the chromosomes of the European domestic cow and those of the wisent cow.

Table 1 summarizes length measurements of the chromosome arms of European domestic cattle and wisent. A statistical treatment of the material of figures shows that the distribution of relative arm lenghts is not significantly different between the two species.

Unfortunately I am unable to report anything about the morphology

TABLE 1. *The distribution of relative chromosome arm sizes from mitotic metaphase plates from two S.L.B cows and a wisent cow.*

Material	Frequences of arms within size classes															n	Mean square
	0.6	0.8	1.0	1.2	1.4	1.6	1.8	2.0	2.2	2.4	2.6	2.8	3.0	3.2	3.4 %		
Cattle, mitotic plate ♀ I			5	10	11	8	5	7	8	4	1	2			1	62	0.274
„ , „ „ ♀ II		1	2	9	14	9	6	10	4	2	3	1		1		62	0.233
Wisent, „ „ ♀		1	7	11	5	9	7	5	5	6	3	3				62	0.300
Total		2	14	30	30	26	18	22	17	12	7	6		1	1	186	0.266

The relative length of a chromosome arm in Tables 1 and 2 is expressed in percentages of the total chromosome length of the complement (The sum of the two chromatide measurements of every chromosome was divided by the total chromatide length and multiplied by 100).

The mean square value for "between cells" is lower than that "within cells".

Fig. 6. European bison or wisent. — Drawing of a mitotic plate from a cow. — ×1800.

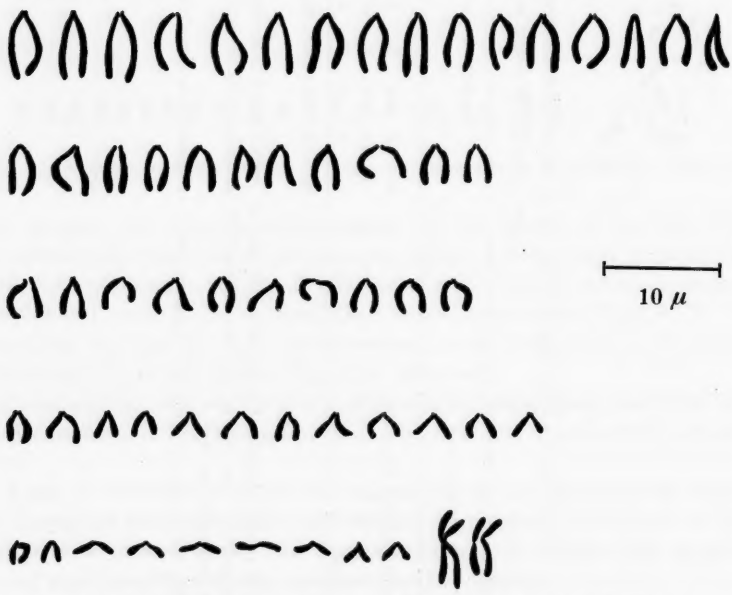

Fig. 7. European bison or wisent. — Idiogram of the plate in Fig. 6.

of the Y-chromosome of the European bison until a male specimen has been studied which would be very desirable. Judging from the situation in cattle the Y ought to be a very small metacentric chromosome.

IV. SOME PHYLOGENETIC ASPECTS OF THE CHROMOSOME COMPLEMENTS IN THE SUBFAMILY BOVINAE

According to WEBER (1928), who adopted the opinion of RÜTIMEYER (1867), the subfamily *Bovinae* is divided into two groups, one more primitive consisting of the genus *Bubalus*, and a second one comprising the four genera *Bibos*, *Bos s. str.*, *Poëphagus* and *Bison*.

PCHAKADZE (1939), who investigated the chromosomes of the domestic buffalo, found a diploid set of 56 rod-shaped elements. MAKINO (1944) found 2n=48 in the closely related water buffalo. 40 of these chromosomes were rod-shaped and 8V-shaped, which makes the number of arms equal to 56. Thus, both authors agree, taking into account that MAKINO, due to his more modern technique, was able to distinguish between medially inserted and telocentric chromosomes. The obvious difference in chromosome number between cattle and buffalo elucidates the fact that attempts to cross these species have been futile (PCHAKADZE 1939).

The available information from carefully investigated species of the second group (*Bibos*, *Bos*, *Poëphagus*, *Bison*) points to a complement uniformly consisting of 60 chromosomes in all species. However, studies of more species are desirable before the final assessment can be made.

Crosses between cattle and wisent have repeatedly taken place in the zoological gardens of Europe with good fertility in the F_1-hybrids. As mentioned, there are no morphological differences between the chromosome sets of the two species.

The genus *Bibos* is, as far as I can ascertain, quite unknown from a chromosomal point of view. The chromosomes of the yak, *Poëphagus grunniens* have been investigated by ZUITIN (1938), who found 2n=60. According to this author (ZUITIN 1935, 1938) crosses between European domestic cattle and yak give rise to fertile females and sterile males. This disturbance may indicate differences in the chromosome morphology.

The chromosomes of the zebu, *Bos indicus*, have been investigated by MAKINO (1944) who claims that their morphology is very similar to that of European domestic cattle. The zebu is the collective name of the native cattle races in Asia and Africa whereas the European domestic cattle races are collectively called *Bos taurus* (WEBER 1928). Both are

related to the aurochs, *Bos primigenius*. The two branches differentiated from this species perhaps as early as the tertiary period. Breeds of *Bos taurus* are now spread all over the world and are not restricted to Europe. Crosses between European cattle and zebu are fully fertile.

The splitting of this species group, which might have started in the tertiary period, does not seem to have interfered to any great extent with the chromosome morphology. Crosses between zebu and yak, carried out in the zoological park at Moscow (ZAWADOWSKY 1931), gave fully fertile females and sterile males, a result similar to the above-mentioned crosses between European domestic cattle and yak. This means that European and Afro-Asiatic cattle are very closely related, having developed a sterility barrier towards the yak.

V. SHEEP, OVIS ARIES L.

Previous works

The first to study the chromosomes of sheep was WODSEDALEK (1922) who found the diploid number to be 33—34 chromosomes. SHIWAGO (1931) found 54 to be the diploid number with a slight variation downwards. KRALLINGER in his paper (1931) says that the diploid chromosome number lies between 50 and 60 but states that he constantly finds 30 bivalents in the first meiotic division. BUTARIN (1935 a and b) studied two kinds of sheep from Siberia, claimed to be different species. In the wild Arkhar sheep he found 2n=54 and 56; in the fat-rumped sheep he found 2n=52 and 54. He says that the actual number probably lies between 54 and 60 in both of them and that the hybrids of the two should be fertile. One of the chromosomes which appears V-shaped he interprets as the X, whereas the Y should be one within the group of small chromosomes.

NOVIKOV (1935) found 2n=60 as the most probable number in hybrids of the wild European mouflon and the domesticated sheep of the merino breed. He found all chromosomes to be rod-shaped and was unable to distinguish the sex chromosomes in the diploid set. BRUCE (1935) confirms his findings of 2n=60 in the merino sheep. PCHAKADZE's (1936) result with the Georgian fat-tailed sheep is in accordance with the foregoing authors' finding 2n=60.

BERRY (1938) found 54 to be the diploid chromosome number. His material was the Rambouillet breed. In a later paper (1941) he could confirm his own findings with another technique. Similar results were obtained by AHMED (1940) in the Leicester breed. MAKINO's (1943)

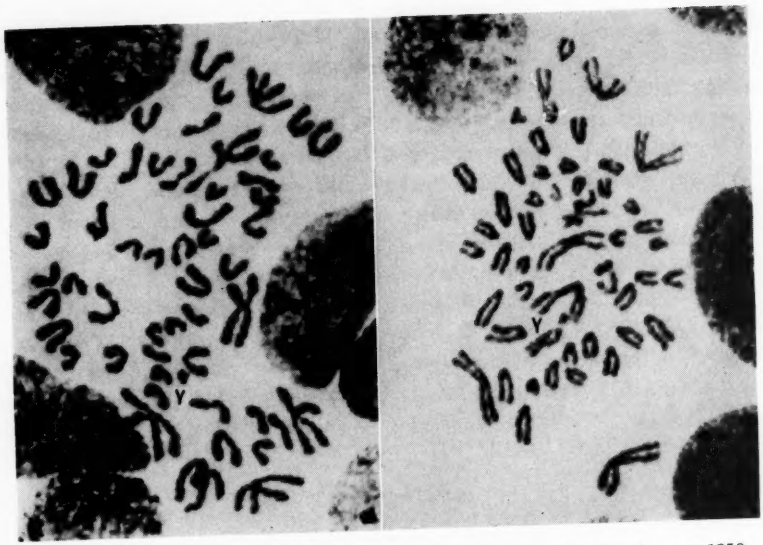

Fig. 8. Sheep. — Mitotic plates from two rams of the Karakul breed. — ×1250.

research in the Merino, Karakul and Corriedale breeds confirms the findings of BERRY, *i. e.* 54 chromosomes in the diploid set.

Thus, the observations of the chromosome number of sheep are contradictory. A group of somewhat earlier authors reported 60 as the diploid number, but contrary to this a group of later investigators claimed 2n to be 54. There is disaggreement not only as to the number but also as to the morphology of the chromosomes. According to those authors who found 2n to be 60 all chromosomes are rod-shaped. BERRY (1938, 1941), AHMED (1940) and MAKINO (1943) who found 54 report some pairs as having a medially located centromere. BERRY (1941) and MAKINO agree in finding three such pairs with a submedian centromere.

WODSEDALEK (1929) claims that the ram lacks a Y-chromosome. AHMED (1940) thinks that the Y-chromosome is subterminally inserted and of medium size. All other authors agree that the Y is a small chromosome, but nobody has been able to see the position of its centromere. There has been some discussion about the morphology of the X-chromosome. SHIWAGO (1931), BRUCE (1935) and BUTARIN (1935 a) place the X among the larger of the medium-sized chromosomes and so does BERRY (1941) whereas AHMED (1940) figures it as a large V-shaped chromosome. In MAKINO's investigation (1943) it is said to be a very long, rod-shaped element.

Own observations

My observations on the chromosome number in sheep are in full agreement with those of SHIWAGO (1931), BERRY (1938, 1941), AHMED (1940) and MAKINO (1943). The diploid chromosome number of the Karakul breed has been found to be 54. Three autosome pairs are large, definitely belonging to a separate size class (Figs. 8; 9; Table 2). They have a submedian centromere. All other autosome pairs have a strictly terminal centromere.

A comparison was made from the figures of Table 2 between the lengths of the twelve chromosome arms composing the three pairs of metacentrics in the sheep complement and the lengths of the 47 remaining chromosomes (the Y is not taken into account). From a statistical analysis of the figures in Table 2 it becomes evident that the lengths of arms composing the metacentrics differ significantly from that of the other ones. The metacentric arms are longer.

The X which is among the terminally inserted chromosomes cannot be definitely identified during mitosis. The Y is the smallest chromo-

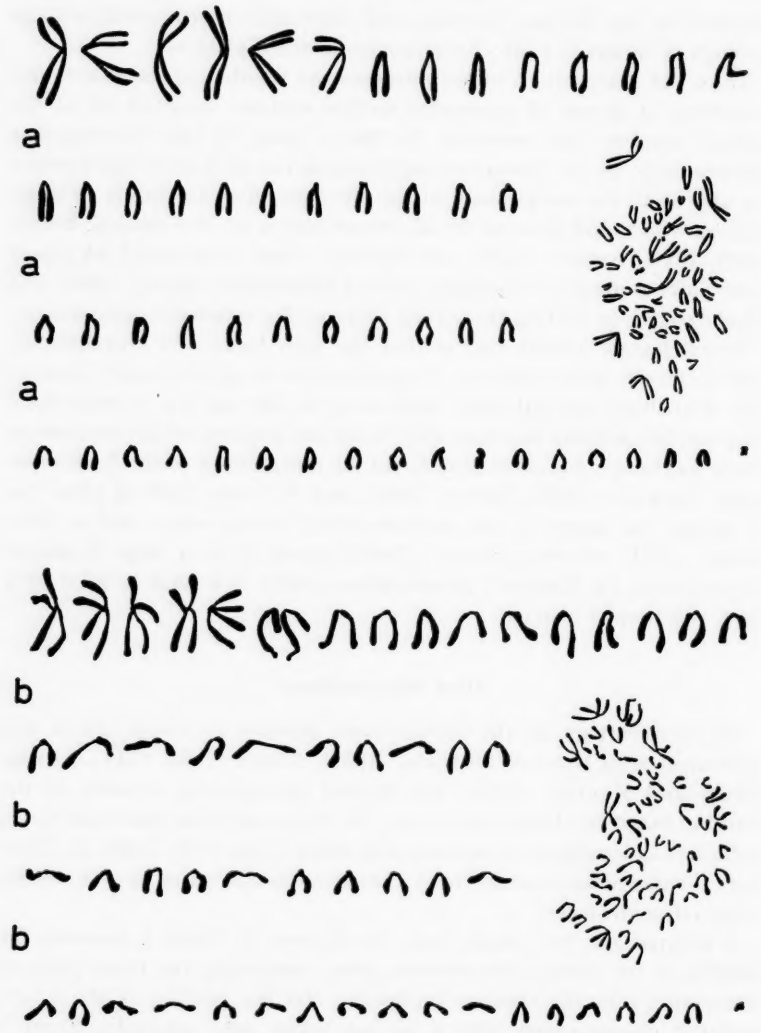

Fig. 9. Sheep. — Idiograms of two rams of the Karakul breed.

some of the complement. It has a median centromere and is positively heteropycnotic during metaphase (Fig. 8). The length of the chromosomes ranges from about 8 to 1 micra.

TABLE 2. *The distribution of relative chromosome arm sizes in mitotic metaphase plates from two rams (See the explanation, Table 1).*

Ram	Kind of arms	Frequences of arms within size classes 0.8	1.0	1.2	1.4	1.6	1.8	2.0	2.2	2.4	2.6	2.8	3.0	3.2 %	n	$\bar{x} \pm m$	t	P
I	Arms from metacentric chromosomes					1	2	2	2		3		1	1	12	2.20±0.14	4.12***	< 0.001
	Arms from other chromosomes	1	10	11	5	6	6	4	3	1					47	1.56±0.06		
II	Arms from metacentric chromosomes					1		1	2	3	3	1		1	12	2.32±0.12	6.16***	< 0.001
	Arms from other chromosomes	3	6	10	10	7	5	5			1				47	1.53±0.02		
	Total	4	16	21	17	15	14	13	6	8	1	1	2		118	1.69±0.05		

VI. DISCUSSION

The discrepancies in chromosome number reported by various authors may be due to the different interpretations of the location of the centromere. If the number of arms is taken into consideration it is evident that the two groups of authors claiming the numbers 54 and 60 respectively are in agreement. Those authors reporting 2n=60 have counted the two arms of one long metacentric chromosome as two chromosomes lying quite close to one another.

Another interpretation would be that the two chromosome numbers reported might be due to racial differences. This, however, seems less likely because different authors have reported both chromosome numbers from the same breed. Most probably the large metacentric chromosomes are phylogenetically new, having arisen according to ROBERTSON's law by some kind of fusion between chromosomes with terminal centromeres.

That the arms composing metacentrics are significantly longer than the other ones may seem somewhat surprising. If the same tendency appears also in the complement of other cavicorn mammals having both medially and strictly terminally located centromeres the question arises if and why relatively large metacentric arms are advantageous in such a complement. Possibly, the structure of the new centromeres of the metacentrics was altered in connection with their origin in such a way that chromosomes happening to be composed of relatively long arms were able to function better than were smaller metacentrics. Thus, the markedly longer total lengths of the metacentrics might be maintained by selection against the size-levelling effect that structural chromosome alterations are supposed to have.

SUMMARY

The number and morphology of the mitotic chromosomes of cattle, European bison and sheep were studied.

Cattle have 2n=60. The sex chromosomes are submedially inserted, the Y is heteropycnotic. Both sex chromosomes can be easily dinstinguished in a good mitotic plate. The autosomes have terminal centromeres.

The complement of the European bison is morphologically similar to that of cattle.

The phylogenetic aspects of the chromosome complements in the sub-

familly *Bovinae* are discussed; the European and Afro-Asiatic cattle are very closely related, having developed a sterility barrier towards the yak.

The sheep have 2n=54. Three pairs of large autosomes are submedially inserted. The X could not be identified. The Y is very small, medially inserted and heteropycnotic.

The arms composing the metacentric autosomes are relatively large, the significance of which is discussed.

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Addendum

After the present paper was already in proof, NAKANISHI and MIZUTANI (1959) communicated some observations on the somatic chromosomes of the lamb. Since their results differ on a few points from those of the present writer, some comments may be called for.

According to these authors most of the autosomes, and the X, are J-shaped (subterminal centromere), only one to three autosome pairs, and the Y being rod-shaped (terminal centromere). In my opinion the Y is clearly metacentric, which seems to be well compatible even with the pictures of NAKANISHI and MIZUTANI. The J- and rod-shaped elements, one of which must be the X, show a gradual length variation from the shortest to the longest, rendering impossible not only the identification of the X, but any mating at all of the chromosomes into homologous pairs. Both in sheep and in cattle, the chromosomes are strikingly similar to mouse chromosomes (*cf.* TJIO and ÖSTERGREN, 1958, Figs. 3; 4; 5 and OHNO, KAPLAN, and KINOSITA, 1958, Fig. 4). After quinoline treatment of certain cytologically favorable mouse ascites tumors, TJIO and LEVAN (1954) were able unequivocally to demonstrate that the centromeres were really terminal in this material, each chromatid ending proximally with two centromeric bodies in tandem (*l. c.*, Fig. 2 *d*; 6 *d*, *f*; 14 *b*). Especially at anaphase, the centromeric bodies very obviously constitute the terminal part of the chromosome, stretched-out towards the pole. If there were an arm on the other side of the centromere, this would be expected to be passively dragged along during the

anaphase movement, thus forming an angle with the rest of the chromosome. No trace of such a second arm was ever seen in critical pictures. In analogy with these observations in the mouse and my own observations especially in cattle, I am inclined to regard the J- and rod-elements of the sheep as having genuinely terminal centromeres, even though tiny knobs of varying size are often seen at the centromeric ends, here as well as in the mouse. These knobs, being especially apparent during the transition from metaphase to anaphase, may indicate that the falling apart of the chromatids is not yet completed. It should be noted that the proximal segments of chromosomes are usually heterochromatic and therefore liable to considerable variation in appearence.

THE HUMAN IDIOGRAM

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(Received September 5th, 1959)

RECENT developments in the chromosome study of cells in tissue culture have brought mammalian chromosomes to a much more detailed morphologic analysis than was possible only a few years ago. Accordingly the chromosomes of man have been repeatedly studied, and idiograms have been published by FORD, JACOBS, and LAJTHA (1958), by TJIO and PUCK (1958), and by CHU and GILES (1959). Recently, during a check-up on the chromosomes of a normal son of a mongoloid mother, we obtained in one fixation an abundance of good metaphases (*cf.* Fig. 1). After the immediate purpose of the investigation was accomplished, viz., a control of the chromosome complement of this person, we were tempted to make a number of complete analyses for the attainment of an idiogram based on the average of a number of plates. Although our results in almost every detail were compatible with the idiograms already published, we think it useful, at the present stage of inquiry, to make a concise report of our findings.

The case history of our patient, a ten year old male child, will be published in connection with the chromosome analysis of his mongoloid mother. A small piece of skin, less than 1 cm^3, was biopsied on May 23, 1959. The sample was immediately immersed into 5 cc of 5 a growth medium prepared according to the formula of MCCOY, MAXWELL, and KRUSE (1959). Within 20 minutes the specimen was cut into many tiny fragments with sharp surgical knives. The fragments were distributed into two T-30 flasks, and a piece of perforated cellophane was used to cover the tissue fragments in each flask. Within a week, outgrowing spindle cells were observed at the periphery of practically every explant. The growth medium was changed every week. Three weeks later the cells were trypsinized (0.2 % trypsin from Nutritional Biochemicals, Cleveland, Ohio) and transferred into T-60 flasks. A second subculturing was made two weeks later. The sample for chromosome study

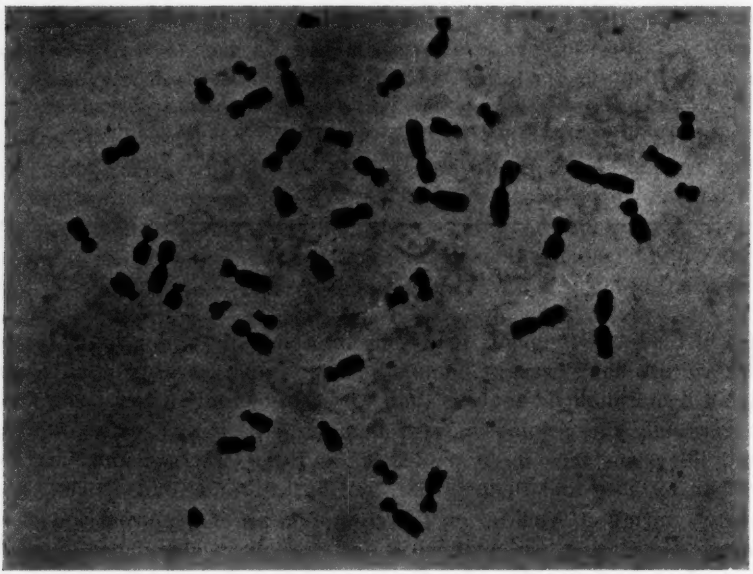

Fig. 1. Photomicrograph of one of the metaphase plates, underlying the idiogram. — × 1500.

was taken from one of these flasks on July 21, 1959. Colchicine solution was added to the growth medium at a final concentration of 1 γ/cc. The cells were incubated in the colchicine medium overnight, treated with a hypotonic solution (1.12 % sodium citrate) for 5 minutes, removed from the flasks, centrifuged, and fixed in 50 % acetic acid. After removal of the acetic acid, the cells were suspended in acetic orcein. Squashes were made from this suspension.

Ten metaphase plates, showing only moderate colchicine contraction, and with all chromosomes lying perfectly in one plane, were drawn with camera lucida, first *in situ* at low magnification, then each chromosome separately at 6000 times magnification. Specific care was taken to draw each chromosome in the center of the viewfield, demarking as exactly as possible the outline of each chromosome arm. The chromosomes were measured on the pencil drawings with a ruler graduated into 0.1 μ.

The results of these measurements are summarized in Table 1. As seen from this Table, and from Figs. 2 and 3, we have grouped the

TABLE 1. *Averages and variation limits for chromosome length (in μ) and arm index in the ten metaphase plates analyzed*

Group	Chromosome number	Chromosome length μ		Arm index	
		Average	Variation limits	Average	Variation limits
I	1	6.8	5.5— 7.9	1.1	1.0—1.2
	2	6.3	5.1— 7.9	1.6	1.5—2.0
	3	5.5	4.3— 6.8	1.2	1.0—1.3
II	4	5.0	4.0— 5.9	2.7	2.3—3.5
	5	4.7	4.1— 5.4	2.6	2.2—3.3
	6	4.5	3.7— 5.4	1.7	1.3—2.3
III	X	4.2	3.7— 4.8	1.6	1.5—1.8
	7	4.1	3.4— 4.8	1.9	1.5—2.8
	8	3.9	3.2— 4.9	1.6	1.1—2.1
	9	3.7	3.0— 4.5	1.8	1.4—2.7
	10	3.6	3.0— 4.2	2.0	1.2—2.7
	11	3.5	2.8— 4.5	2.2	1.5—3.1
	12	3.3	2.5— 3.9	1.7	1.5—2.3
IV	13	2.4	2.0— 3.0	2.4	1.9—3.3
	14	2.0	1.5— 2.4	2.6	1.9—3.5
V	15	2.5	2.0— 3.0	1.4	1.1—1.9
	16	1.9	1.4— 2.3	1.2	1.0—1.6
	17	1.7	1.2— 2.1	1.2	1.0—1.7
VI	18	3.0	2.6— 3.6	4.6	3.3—6.2
	19	2.8	2.4— 3.4	4.7	3.2—8.0
	20	2.6	2.2— 3.2	5.0	3.8—7.3
VII	Y	1.4	1.2— 1.7	4.9	3.7—6.5
	21	1.3	0.9— 1.7	2.0	1.4—5.5
	22	1.1	0.8— 1.4	2.5	1.2—4.5
Total per cell		157.1	134.2—178.4	—	—

chromosomes into seven categories to promote easy identification. Broadly speaking, our grouping is the same as that used by earlier writers. The average chromosome size varied from 1.1 μ to 6.8 μ with the actual extreme individual values ranging from 0.8 to 7.9 μ. It was noted that homologous chromosomes within the same cell may show a considerable difference in length, as exemplified in Table 2 on the measurements for the longest chromosome, nr 1. The fact that, within

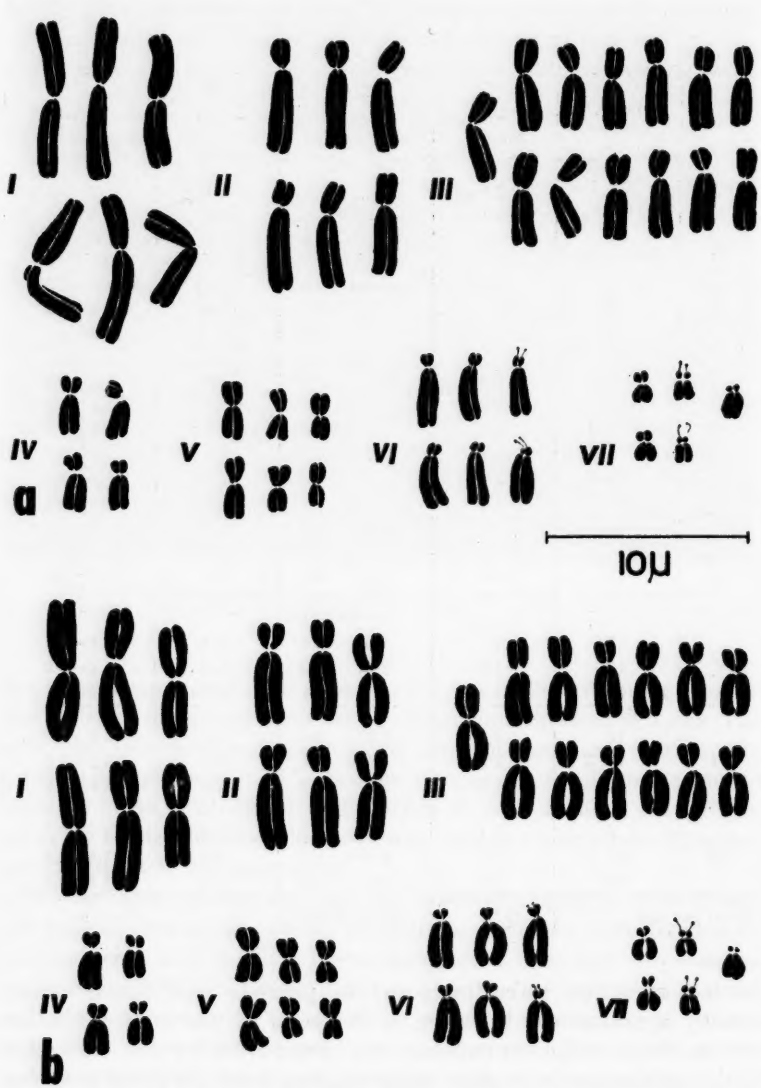

Fig. 2. Two of the metaphase plates, separately drawn. — × 3100.

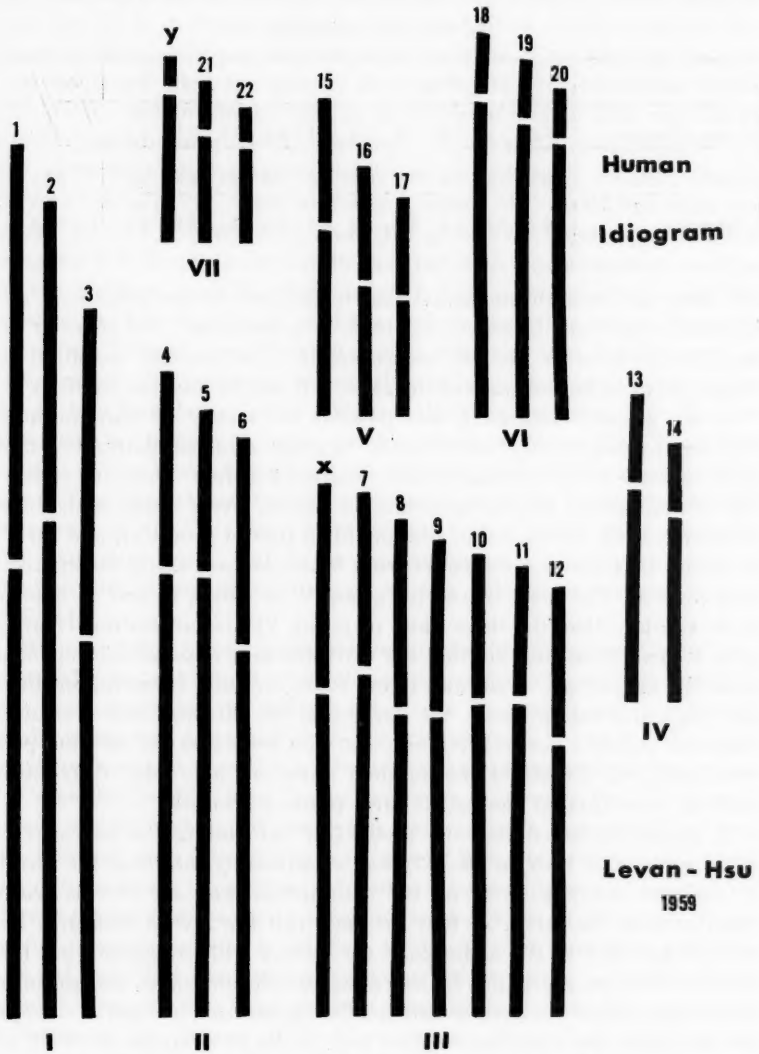

Fig. 3. Average idiogram, based on ten metaphase plates.

TABLE 2. *The length (in μ) of the two partners of Chromosome 1 in the ten cells analyzed*

Cell Number	1	2	3	4	5	6	7	8	9	10	Average
Longer Partner	7.3	7.4	7.3	7.0	7.9	7.1	6.9	6.5	6.5	5.8	6.98
Shorter Partner	6.9	7.4	7.2	6.9	6.7	6.6	6.3	6.1	5.8	5.5	6.54
Difference	0.4	0.0	0.1	0.1	1.2	0.5	0.6	0.4	0.8	0.3	0.44

the same cell, two chromosomes that to the best of our judgement are identical may vary in length by 15 % is a reminder that there is an uncontrollable error an any measurement. The average variation in length between homologues of the same cell was in this case about 6 %.

In the present material it was possible to classify all chromosomes into seven groups with great certainty. In addition, all the pairs, or certain pairs of some groups, could be individually identified. Thus, the following chromosomes are recognizable in almost every good metaphase plate: 1,2, 3, 13, 14, 15, 21, 22, the satellited pair of group VI, and the Y. In group II, 4 and 5 are distinct from 6, but are not easily distinguishable *inter se*. The same is true for group V, in which 16 and 17 appear to be similar. Also, the three pairs of group VI are not distinguishable, as is evident from the fact that the satellited pair is sometimes claimed to be the longest one (TJIO and PUCK, 1958), at other times the medium one (CHU and GILES, 1959). We found that the satellited pair was most often the smallest one in the group, but in a few cases the medium pair was satellited. Evidently the random variation may blur differences such as those present among the three pairs of group VI.

It should be noted that the method of calculating the averages in cases such as nr 4—5, or 16—17, may significantly influence the result. If averages are calculated on the assumption that one pair is really smaller than the other, so that for each cell the lowest measures are always referred to the same pair, the values will exaggerate the difference between the pairs. If, however, the chromosomes are assumed to be alike so that the greater and smaller values are recorded at random for the pairs, the same set of data may result in averages showing no difference.

The 13 chromosomes of group III are the most difficult ones to identify. However, an identification would be especially desirable, because this group contains the X chromosome. FORD, JACOBS, and

LAJTHA (1958) indicate in their idiogram that these chromosomes, including the X, are unidentifiable. TJIO and PUCK (1958) mention that many of these pairs are difficult to differentiate. They identify X, however, as larger than any pair of their group IV (corresponding to our group III), but of similar size as nr 6. According to CHU and GILES (1959) "chromosomes 9—12 represent the most difficult group of all for individual chromosome identification, although all are clearly S-types" (l. c., p. 72). Their nr 7 and 8, which also would belong to our group III, are M-types, with about the same arm index as nr 6. They consider X to be of similar length as 5, but with a more median position of the centromere. Thus, the shape of their X is similar to that described by TJIO and PUCK, and the size is somewhat larger. Recently LEJEUNE, TURPIN, and GAUTIER (1959) conclude that the X is one of the smaller chromosomes within this group. They divide our pairs 6—12 into four larger pairs with less distal centromere, M_1—M_4, and three smaller pairs with more distal centromere, Md_1—Md_3. Their X is similar in size to the latter ones but with a somewhat less distal centromere. They make tho following reservation as to the possibility of identification of the X: "Son identification est possible chez l'homme par exclusion après réalisation des autres paires, mais son identification directe sous le microscope est très délicate et peu sure" (l. c., p. G. 44).

We found that in our material unequivocal identification of any specific pairs, or of the X, in group III was impossible. The length variation was continuous, and the difference between the longest and the shortest in the group was too small (3.3—4.2 μ in average; 2.5—4.9 μ in actual measurements) to permit the selection of any characteristic arm index for a certain length class. It was very easy to arrange the 13 chromosomes of each cell into 6 reasonable pairs plus an unpaired X; it was felt however, that this pairing was of very little value since any one of the chromosomes is capable of forming a pair with any of the others. It seems somewhat easier to form the six pairs out of group III if one of the largest members is selected as the X. This conforms with the ideas of TJIO and PUCK and of CHU and GILES; but in our opinion the identification of the X in somatic metaphases cannot be considered definitive as yet, for any of the other chromosomes of group III is just as eligible. In view of these difficulties, it would be highly desirable for the heterochromatic properties of the X, showing up at interphase, to be made visible during metaphase also. Certain pretreatments to achieve this end have recently been indicated by YERGANIAN (1959) and

by OHNO (personal communication). The possibility of such pretreatments in human material should be investigated.

One of the longest chromosomes of the group was tentatively taken out as X. Its arm index varied from 1.5 to 1.8. In seven cases it was actually the longest chromosome of the group, in three cases the longest chromosome was of such an apparently different arm index that another chromosome had to be picked for X. The six pairs of group III were made up mainly on the basis of their arm index. Unlike TJIO and PUCK, who claim a gradual increase in arm index with falling chromosome length all through the autosomes of group III (1.3—1.5—1.9—2.4—2.8—3.1), we found the highest indices in pairs 10 and 11 (more than 2.0), while the shortest pair 12 seemed to be more equal-armed (index 1.7).

Our observations within group VII indicate that its three members can be securely identified. This may be of significance in relation to the study of human trisomics since the mongoloids are trisomic for a small autosome, either 21 or 22. We agree with TJIO and PUCK that the longest chromosome of the group is the Y. It is also the one with the highest arm index. The two pairs, nr 21 and 22, differ in that the smaller one has a satellite. We did not include the satellite in our measurements, since its connection thread varied considerably in length. Even when the satellite is not visible, nr 22 often shows remnants of a nucleolus on its shorter arm, and is therefore usually identifiable.

Our measurements, in comparison with those of TJIO—PUCK and CHU—GILES, are summarized in Table 3. It is evident from this table that the results are, broadly speaking, identical in three cases, the differences being well within the unavoidable error limits. Evidently, human chromosome morphology can be described with highly reproducible results. For all practical purposes, the classification of the chromosomes into categories is reliable. There are some minor differences between earlier idiograms and the present one. For example, the longest chromosomes were somewhat shorter in our case. Generally, our arm indices are somewhat lower (lower in 15 cases; equal in 9 cases; higher in no cases). The latter difference is especially pronounced in group VI. We found the short arm of the chromosomes in this group often to be narrower than the normal chromosome width; so our length measurements here mean a smaller chromosome volume than usual. It is easy to see that a different fixing action may reduce these short arms into small spheres, which would result in arm indices around 10, as has been earlier reported.

TABLE 3. *Comparison between our idiogram and two earlier idiograms by* TJIO *and* PUCK *(1958) and* CHU *and* GILES *(1959)*

(Chromosome length expressed as percentage of total autosomal length per cell)

Group		I			II			III							IV		V			VI			VII		
Pair		1	2	3	4	5	6	X	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Y	21	22
Chromosome length	T. & P.	9.6	8.7	7.4	6.8	6.2	5.8	6.3	5.0	4.7	4.7	4.6	4.6	4.5	3.1	2.6	3.4	2.3	2.2	3.7	3.4	3.1	2.0	1.9	1.8
	C. & G.	9.5	9.2	7.6	6.6	6.1	5.9	—	5.5	4.9	4.9	4.7	4.6	4.5	3.2	2.6	2.8	2.3	2.0	3.6	3.4	3.3	—	1.6	1.3
	Present	8.9	8.3	7.3	6.6	6.2	5.9	5.5	5.4	5.1	4.9	4.7	4.6	4.4	3.1	2.6	3.2	2.5	2.2	3.9	3.7	3.4	1.9	1.7	1.4
Arm index	T. & P.	1.1	1.6	1.2	2.9	3.2	1.7	1.9	1.3	1.5	1.9	2.4	2.8	3.1	2.8	3.8	1.8	1.4	1.3	8.0	7.3	10.5	—	3.7	3.3
	C. & G.	1.1	1.5	1.2	2.9	3.2	1.8	2.1	1.9	1.7	2.4	2.3	2.1	3.1	2.1	3.8	1.6	1.9	1.3	9.5	9.7	11.9	5.0	6.8	6.0
	Present	1.1	1.6	1.2	2.7	2.6	1.7	1.6	1.9	1.6	1.8	2.0	2.2	1.7	2.4	2.6	1.4	1.2	1.2	4.6	4.7	5.0	4.9	2.0	2.5

Acknowledgements. — Financial support by grants C-4409 from the National Cancer Institute of the United States Public Health Service and G-5645 from the National Science Foundation is gratefully acknowledged. Our thanks are also due to Dr. R. J. BLATTNER, Baylor University College of Medicine, Houston, for supplying the material for this investigation.

SUMMARY

An idiogram is given for the somatic chromosomes of a human male based on measurements of ten metaphase plates from a third subculture of skin cells. The present idiogram conforms closely with those recently published by FORD, JACOBS, and LAJTHA (1958) by TJIO and PUCK (1958), and by CHU and GILES (1959). The certainty with which individual pairs may be identified is considered.

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BRIEF REPORTS

GERT BONNIER: Note on the relative sensitivity to irradiation damages in homozygous and heterozygous *Drosophila melanogaster* with regard to their capacity of producing offspring.

(Received October 17th, 1959)

In an experiment, still in progress, on irradiated populations of *Drosophila melanogaster*, different viability tests are made. The present note will only deal with one of these tests, performed during the earlier generations of the experiment. The note is confined to the presentation of the material whereas the discussion of the problems involved must await the concluding of the experiment.

With flies from the wild type stock "Canton S" a number of populations were started. In an analysis of 89 second chromosomes from this stock 12 were found to be lethal; it included hence many heterozygosities. One type of populations (A) was started from flies taken directly from this stock. Another type of populations (B) was started from a strain which was made homozygous for the three major chromosomes which all emanated from chromosomes within the Canton S stock. All flies were given an x-ray dose of 1 500 r per generation. The length of each generation was exactly three weeks, and no overlaps occurred. Prior to irradiation a number of females and males were randomly collected from the populations. No regard was paid to virginity of the females. The flies were put into vials with food, one female and two males per vial. By transferring the flies to new vials the females did oviposit during two consecutive periods, the length of each being exactly 48 hours. The adult progeny from the two periods was counted; if a female died within a period her progeny was excluded from the counts. An exactly similar procedure was followed with flies collected from the populations just after the populations were irradiated.

These tests were not made with flies from all populations during the earliest generations. The tables, therefore, include results from females, the ancestry of which had got from 6.0 to 10.5 kiloroentgens. Table 1 shows the averages of unirradiated females (x) and irradiated females (y). The progeny from females of the homozygous populations B was smaller than that from females of the heterozygous populations A, but the table also indicates that the relative decrease, when the offspring of irradiated females is compared with that of unirradiated females, is greater in B than in A. To test this further the ratios of the offspring of irradiated females to that of unirradiated ones (y/x) were computed as shown in Table 2. The irradiated females were three days older than the unirradiated ones at the beginning of the 48 hour periods. We do not know if the egglaying rhythms are different in A and B, but if so, the frequency of x-ray induced mutations could possibly be different in A and B. If one compares the number of progeny during the first 48 hour period for irradiated females with that during the second 48 hour period for unirradiated females

TABLE 1. *Average offspring from two consecutive 48 hour periods of oviposition of flies which were themselves unirradiated (x) and irradiated (y).*

The averages refer to tests of single females. *n*, number of females tested. Suffixes 1 and 2 refer to first and second 48 hour period. The dose is given in kiloroentgens.

Dose	Unirradiated flies				Irradiated flies			
	A		B		A		B	
	n1	*x1*	*n1*	*x1*	*n1*	*y1*	*n1*	*y1*
6.0	52	76.6±5.7	57	50.9±3.7	54	67.7±4.2	48	4.7±2.4
7.5	46	107.0±7.1	49	26.5±2.7	52	40.1±3.4	35	5.0±1.0
9.0	54	95.6±4.1	45	45.0±3.9	57	48.7±3.6	53	13.9±1.5
10.5	59	88.3±4.9	55	54.5±3.6	52	46.2±3.1	51	11.8±1.3
	n2	*x2*	*n2*	*x2*	*n2*	*y2*	*n2*	*y2*
6.0	51	109.5±5.7	57	35.5±3.1	49	77.6±5.3	42	12.3±1.6
7.5	45	98.2±7.4	43	23.9±3.4	50	57.3±4.0	29	0.8±0.3
9.0	53	102.5±5.3	40	43.9±4.8	54	62.6±4.8	49	10.8±2.1
10.5	59	76.2±6.2	55	47.2±4.3	49	59.9±4.1	45	9.8±2.1

TABLE 2. *Ratios of average offspring between irradiated and unirradiated females, y/x.*

The figures show ratio within A less ratio within B, thus in the first line of $y1/x1$, $\frac{67.7}{76.6} - \frac{4.7}{50.9}$.

Dose	*y1/x1*	*y2/x2*	*y1/x2*
6.0	0.79±0.098	0.36±0.082	0.49±0.084
7.5	0.18±0.058	0.55±0.061	0.20±0.068
9.0	0.20±0.062	0.36±0.079	0.16±0.066
10.5	0.30±0.053	0.58±0.097	0.36±0.073

the maximal age difference will be one day. This comparison is, therefore, also shown in Table 2.

There are several causes of variation due to environment which are not under control and which are not eliminated from the standard errors of the tables. But even if the figures of Table 2 should not be taken at their face values, all differences are going in the same direction. Moreover, all the statistical significances are, as computed, of such a high magnitude, that it seems impossible to escape from the conclusion that the differences observed are based on biological truths. Granting that this is so, it seems inevitable to conclude that homozygous (or more correctly, very little heterozygous) flies, in their capacities to produce offspring, are relatively more sensitive to irradiation than are heterozygous females.

Institute of Genetics, University of Stockholm, Sweden.

TILL MEDARBETARNA

Manuskript — helst *maskinskrivna* — torde insändas till redaktionen (Genetiska Institutionen, Lund) i fullt tryckfärdigt skick. De böra vara *noga genomsedda* för undvikande av ändringar mot manuskriptet. Obs. kommateringen! Korrektionskostnaderna betalas av författaren. Korrektur ställes till redaktionen. Direkt förbindelse mellan författaren och tryckeriet tillåtes icke.

Personnamn sättas med KAPITÄLER. De markeras i manuskriptet med en våglinje. Latinska namn på växter och djur samt ord och satser av särskild vikt *kursiveras* (enkel understrykning).

Figurer numreras med arabiska siffror. Figurförklaring (på avhandlingens språk) torde insändas *samtidigt* med illustrationsmaterialet.

Tabeller åsättas arabiska siffror och förses med kort rubrik.

Citerade arbeten samlas i en litteraturförteckning. I texten hänvisas till denna genom angivande av författare och årtal. Har en författare utgivit flera publikationer under samma år, tilläggas efter årtalet små bokstäver (a, b, c, etc.). Samma beteckningssätt användes i litteraturlistan, vilken uppställes i alfabetisk ordning efter författarna och under dessa i kronologisk följd. Inga litteraturhänvisningar få göras genom fotnoter. Överhuvudtaget så få noter som möjligt!

Avhandlingarna skola vara skrivna på engelska, tyska eller franska. Det är önskvärt, att uppsatser på tyska och franska åtföljas av en resumé på engelska. Översättningar, som ombesörjas av redaktionen, bekostas av författaren.

Åt varje författare lämnas 100 fria separat. Avhandlingar på ett ark och däröver förses gratis med särskilt omslag. Till ett pris av 25 kr. pr 100 st. lämnas, om så önskas, omslag till mindre uppsatser. Större antal särtryck fås till självkostnadspris. *Anteckning om eventuella extraseparat göres å första korrekturet.*

Hereditas is open to Scandinavian writers only (and to foreigners working at Scandinavian institutes).

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